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A STUDY OF THE EFFECT OF PROCAINE INTRAVENOUSLY ON HISTAMINE AND ALLERGIC PHENOMENA

JOHN D. ARCHER*

State and Wangensteen (1946) published results of clinical use of procaine intravenously to combat various types of allergic reactions. They traced the history of the use of procaine intravenously beginning with Bier, who in 1909 applied tourniquets to limbs, made them bloodless and anesthetized them with procaine intravenously. In 1942, Lundy successfully used 20 cc. of 0.1% procaine to relieve the pruritis of jaundice. In 1941, Leriche used procaine intravenously against various vasomotor disorders. In 1943, Gordon reported its use to abolish the pain in dressing burns. Breton and Gedoux were early to use procaine intravenously against allergic phenomena; 0.1 mg. per Kg. prevented and combated drug idiosyncrasies but not anaphylactic shock in animals nor serum sickness in man. Durieu, *et al.* (1946) reported that Dos Ghali daily injected 5 to 20 cc. of procaine intravenously to chronic asthmatics and gave lasting improvement to 50% and temporary improvement to 30%.

State and Wangensteen (1946) used procaine intravenously in doses of one gram in 500 cc. normal saline and reported that one or more doses gave fast and complete relief from the manifestations of delayed serum sickness in 10 of 16 patients. The manifestations included maculopapular and purpuric rashes, urticaria, fever, chills, myalgia, arthralgia, lymphadenopathy, sore throat, nausea, vomiting, malaise and anorexia. Four of the others obtained temporary or partial relief. One patient obtained relief from the dyspnea and urticaria of an immediate anaphylactoid reaction following the injection of bovine albumin. Six of seven patients with urticaria were reported relieved by procaine intravenously; two of these were urticarias produced by a known sensitizing agent. The drug

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was found ineffective in a patient with chronic asthma and in another with rheumatoid arthritis.

Paradoxically, procaine itself is well known as a sensitizing agent. It commonly produces dermatitis (Goodman, 1939; Orland, *et al.*, 1944; Robinson, *et al.*, 1945; Robinson, 1935; Cipollaro, 1936) and has been known to produce death by anaphylactoid reaction even though given intradermally in therapeutic doses for local anesthesia (State and Wangensteen, 1946).

State and Wangensteen (1946) suggest several possible modes of action of procaine against allergic phenomena: (1) an antihistaminic action, (2) an anticholinergic action, (3) an epinephrine potentiating action, and (4) a direct action on cells, specifically an anesthetizing of pain fibers from muscles and joints.

Structure and Fate.—Procaine is the ester of para-aminobenzoic acid and diethylaminoethanol. Having a trialkylamino group, it is grossly similar to the dimethylaminoethyl derivatives antagonizing histamine. It is administered usually as its hydrochloride salt. In the blood stream, it hydrolyzes rapidly to *p*-aminobenzoic acid and diethylaminoethanol. Some of the action attributed to procaine intravenously is probably the result of the diethylaminoethanol. Most of *p*-aminobenzoic acid is excreted in the urine free or conjugated. About one-third of the diethylaminoethanol is excreted; the fate of the remaining two-thirds is unknown. Similar results follow administration of *p*-aminobenzoic acid and diethylaminoethanol separately. In two human subjects receiving two grams of procaine intravenously in less than two hours, the highest concentration of procaine found in the plasma at any time was 0.35 mg. per liter; the highest concentration of diethylaminoethanol was 3.7 mg. per liter; the highest concentration of *p*-aminobenzoic acid was 15.1 mg. per liter (Brodie, *et al.*, 1948).

History of Experimental Reports.—Brunati (1939) reported that 5 mg. of procaine protects guinea pigs from a lethal dose of histamine. Frommel, *et al.* (1947), confirmed this work and also found that procaine inhibits contractions of guinea pig ileum produced by histamine *in vitro*. Hazard and Corteg-

giani (1943) reported that procaine inhibits histamine-induced spasms of isolated, perfused guinea pig lung.

Recent Experiments

I felt that the previously demonstrated antagonism of procaine to histamine on smooth muscle preparations was perhaps only a musculotropic action instead of a specific antihistaminic action and that the observations *in vivo* deserved further investigation (Archer, *et al.*, 1951).

The first approach was to investigate whether procaine affected the release of histamine by blood cells. Serial concentrations of procaine-HCl were added to samples of blood from a rabbit sensitized with Freund's antigen (Achard and Aynaud, 1909), and the samples were carried through Katz' (1940) method for studying the release of histamine. Aliquots of 2 cc. plasma were then carried through the method of McIntire, *et al.* (1947), for purifying histamine, and the eluates were bioassayed for histamine by Code's (1937) modification of the technique of Barsoum and Gaddum. The results are shown in Table 1.

TABLE 1

Constituents of Reaction			Histamine found in
Blood	Procaine HCl	1:100 Egg White	0.5 cc. of eluate
2.6 cc.	.....	....	0.10 γ
2.6 cc.	1.0%	....	0.02 γ
2.6 cc.	.....	1 cc.	0.18 γ
2.6 cc.	.....	1 cc.	0.20 γ
2.6 cc.	1.0%	1 cc.	0.03 γ
2.6 cc.	0.1%	1 cc.	0.05 γ
2.6 cc.	0.01%	1 cc.	0.15 γ
2.6 cc.	0.001%	1 cc.	0.20 γ

The suggestion by the results of Table 1 that the release of histamine was inhibited by the higher concentrations of procaine became unconvincing when it was found that a sample containing 0.5 cc. of No. 5 added to 2.0 gammas of histamine caused a contraction of the guinea pig ileum equivalent only to 1.0 gamma of histamine alone. Furthermore, 3.0 gammas of histamine added to 3.0 mg. of procaine caused a contraction equivalent only to 0.7 gamma of histamine alone. Obviously some procaine was coming through the purification procedure

to contaminate the eluate and to interfere with the bioassay. To determine the amount and evaluate its effect, 60 mg. of procaine were taken through the purification method and the resulting eluate was compared with standards by the method of Bratton and Marshall (1939) for determining sulfonamides. 2.8 mg., or 4.7% of the procaine were present in the eluate. From this result, it was inferred that in the experiment concerning the release of histamine, the sample containing 1% procaine gave an eluate containing approximately 0.06 mg. of procaine. Aliquots of 2.5 cc. were then bioassayed, each containing this quantity of procaine plus known quantities of histamine equivalent to those suspected of being present in the experiment concerning the release of histamine. The results are shown in Table 2.

TABLE 2

Histamine in 0.5 cc. of the sample	Procaine in 2.5 cc. of the sample	Histamine causing contraction equal to 0.5 cc. of the sample
0.1 γ	0.06 mg.	0.01 γ
0.2 γ	0.06 mg.	0.03 γ

The results of Table 2 indicate that the amounts of procaine contaminating the eluates in the prior experiment were sufficient to cause the superficially apparent inhibition of the release of histamine. It also should be mentioned that the lowest concentration of procaine used, which completely failed to give any indication of inhibiting the release of histamine, was prodigiously in excess of the blood levels of procaine and its hydrolytic products in therapeutic use (Brodie, *et al.*, 1948). It can be concluded that procaine probably does not inhibit the release of histamine by blood cells.

An investigation was made concerning any possible effect of procaine locally on histamine wheals in the human forearm. The results shown in Table 3 fail to indicate that it gives any protection.

The effect of procaine locally on an antigen-antibody reaction independent of any histamine phenomenon seemed worthy of investigation. The shaved skin of rabbits sensitized with Freund antigen (Achard and Aynaud, 1909) was used. Rab-

TABLE 3

Site of injection	Constituents of injection: 0.9% NaCl	Histamine HCl in 100γ/cc. sol.	Procaine HCl 2%	Size of wheal after 10 min.
Upper forearm	0.10 cc.	2 γ	.....	4.8 x 6.8 cm = 32.6 cm^2
Upper forearm	.....	2 γ	0.1 cc.	5.5 x 6.1 cm = 33.6 cm^2
Lower forearm	0.10 cc.	2 γ	.....	3.5 x 4.4 cm = 15.4 cm^2
Lower forearm	.....	2 γ	0.1 cc.	3.5 x 4.3 cm = 15.1 cm^2
Lower forearm	0.12 cc.	...	.....	1.0 x 0.9 cm = 0.9 cm^2

bits were suitable animals for the experiment, as histamine locally in relatively high concentration produces no wheal in their skin (Sollmann and Pilcher, 1917; Darsie, *et al.*, 1945). Lesions were produced by injecting intracutaneously and into the same site 0.1 cc. of 1% egg white and 0.1 cc. of 2% procaine. As a control, each rabbit received an injection of 0.1 cc. of 1% egg white and 0.1 cc. of normal saline. The lesions were observed after three hours. Table 4 shows that the reactions in four rabbits were markedly more severe where the procaine was present.

TABLE 4

Animal	Area of lesion produced by 0.1 cc. of 0.01% egg white and 0.1 cc. of 0.9% NaCl	Area of lesion produced by 0.1 cc. of 0.01% egg white and 0.1 cc. of 2% procaine HCl
1.....	1.0 cm^2	2.3 cm^2 (necrotic center present)
2.....	1.1 cm^2	2.2 cm^2 (necrotic center present)
3.....	No significant reaction	No significant reaction
4.....	4.0 cm^2	5.1 cm^2 (necrotic center present)
5.....	1.4 cm^2	1.4 cm^2
6.....	No significant reaction	No significant reaction
7.....	1.2 cm^2	2.1 cm^2 (necrotic center present)

The effect of partial anesthesia on the histamine wheal not in the proximate presence of procaine was investigated by partially blocking the nerve supply to a part of the hand of two human subjects. The wheals, produced by 5 gammas of histamine, were compared with control wheals in the similar site on the other, unanesthetized hand. Table 5 shows that only moderate differences were noted; in one subject the partially anesthetized skin had the slightly larger wheal; in the other subject it had the smaller one.

TABLE 5

Subject	Area of wheal in unanesthetized area	Area of wheal in partially anesthetized area
1.....	6.9 cm^2	5.6 cm^2
2.....	4.0 cm^2	5.0 cm^2

Through the cooperation of Dr. A. W. Harrison, I had the opportunity to study the influence of procaine on histamine-induced contractions of human smooth muscle. The normal upper lobe of a lung removed because of carcinoma of the lower lobe was studied by the technique developed by Gilbert (1951). A warm solution of gelatin in Locke-Ringer solution was poured into the bronchial tree of the freshly removed segment. This was allowed to solidify in Locke-Ringer solution kept in an ice bath. Slices were made perpendicular to bronchioles, and the bronchiolar contractions and relaxations were measured under the dissecting microscope with a micrometer. The results of the direct application of histamine to the segments of bronchioles and the subsequent application of procaine, diethylaminoethanol or tripelennamine are shown in Table 6.

TABLE 6

Original diameter of bronchiole	Histamine administered	Resulting diameter of bronchiole	Subsequent compound administered	Final diameter of bronchiole
36.....	1 mg.	33	100 mg. % procaine HCl	32
27.....	2 mg.	23	1000 mg. % procaine HCl	25
35.....	3 mg.	31	100 mg. % procaine HCl	32
20.....	1 mg.	15	10 mg. % procaine HCl	16
32.....	1 mg.	22	20 mg. % procaine HCl	27
16.....	1 mg.	0	100 mg. % procaine HCl	0
30.....	1 mg.	27	100 mg. % procaine HCl	27
31.....	2 mg.	26	5 mg. % tripelennamine	30
12.....	2 mg.	0	5 mg. % tripelennamine	12
27.....	3 mg.	23	20 mg. % diethylaminoethanol	23
6.....	2 mg.	3	50 mg. % diethylaminoethanol	4
11.....	2 mg.	6	20 mg. % diethylaminoethanol	10
40.....	3 mg.	28	20 mg. % diethylaminoethanol	28

(Note: Many slices failed to react to histamine; these results are not recorded.)

Procaine and diethylaminoethanol were irregularly and only partly effective in relieving the histamine-induced contractions.

The concentrations necessary for any effect were tremendous in comparison with therapeutic blood levels. The bronchioles became non-reactive before adequate data were obtained, but the impression was that tripelennamine was effective to a greater degree and in much smaller concentrations than were procaine or diethylaminoethanol.

The effect of procaine locally on the inflammatory reaction of the skin caused by a primary irritant seemed possibly related, at least superficially, to the use of procaine intravenously against skin allergy. Morita (1926) reported that procaine intravenously protected rabbits' skin from inflammation by mustard oil, the reactions being observed the next day. In the present experiment, the shaved skin of each of four rabbits was injected with 0.2 cc. of 1.0% procaine-HCl in one site and with 0.2 cc. of normal saline in another site. Xylene was then applied to the sites as an irritant, and 5 cc. of 1% trypan blue was injected into the ear vein. In every rabbit, the escape of the dye was considerably inhibited for about 30 minutes in the procainized areas as compared with the control areas. This phenomenon was interpreted as being somewhat of an anti-inflammatory action, either by opposing local capillary permeability or dilatation. The significance of this experiment is vague; intravenous procaine, of course, is considered to be a vasodilator (State and Wangensteen, 1946).

The final investigation at this point has been to study with puppies the acute toxicity of lethal doses of histamine intravenously as influenced by large doses of procaine. Sollmann and Hanzlik (1939) report a lethal dose as 30 mg. of histamine base per Kg. As shown in Table 7, we injected 30 mg. per Kg. of histamine-HCl to seven puppies, 30 mg. of procaine-HCl per Kg. to four others and a mixture of 15 mg. per Kg. of each to twelve. The solutions contained 30 mg. per cc. of normal saline and were pushed rather rapidly into a leg vein.

The animals receiving only histamine fell prostrate, defecated, urinated, retched, salivated copiously and developed labored respiration. Those receiving only procaine developed brief tonic and clonic convulsions and then arose within a few

TABLE 7

Animal	Weight in Kg.	Histamine in mg./Kg.	Procaine in mg./Kg.	Died	Lived
1.....	1.15	..	30	..	+
2.....	3.35	..	30	..	+
3.....	4.20	..	30	..	+
4.....	3.60	..	30	..	+
5.....	0.40	30	..	..	+
6.....	1.10	30	..	..	+
7.....	3.37	30	..	..	+
8.....	2.90	30	..	+	..
9.....	2.25	30	..	+	..
10.....	2.98	30	..	+	..
11.....	1.80	30	..	..	+
12.....	2.10	15	15	+	..
13.....	2.81	15	15	+	..
14.....	2.21	15	15	+	..
15.....	3.90	15	15	+	..
16.....	1.15	15	15	..	+
17.....	2.42	15	15	+	..
18.....	4.57	15	15	+	..
19.....	1.60	15	15	+	..
20.....	2.30	15	15	+	..
21.....	2.70	15	15	+	..
22.....	3.10	15	15	+	..
23.....	3.15	15	15	+	..

minutes. Those receiving the combined, half-sized doses developed the clonic and tonic convulsions, defecated, urinated, retched, salivated copiously and soon developed Cheynes-Stokes respiration. The recoveries noted in Table 7 are not reported as twenty-four hours survivals as is customary in acute toxicity studies; a puppy that got up, breathed normally, walked normally and played within an hour or two is reported as a recovery. Except for No. 5, all of these animals receiving histamine did die 6 to 12 hours later, apparently from intestinal hemorrhage. The animals reported in the table as dying apparently did so from respiratory failure without ever getting up or showing significant signs of recovery. So lethality in this experiment refers only to respiratory death. Except for No. 23, all eleven deaths from the combined drugs occurred within less than an hour, usually within less than twenty minutes. The three from the larger dose of histamine alone occurred between one and two hours after the injection. It was impressive that the animals receiving the combined drugs appeared more drastically ill than those receiving the larger dose of either drug alone.

The results lead to the conclusion that procaine and histamine in the amounts given and with the method used potentiate the immediate toxicity of one another. One-half of a non-lethal dose of procaine together with one-half of less than the LD_{50} of histamine killed 11 of 12 dogs and killed them faster and more violently. It should be mentioned, of course, that the administration of procaine in this experiment by no means resembles its therapeutic administration against allergic phenomena. But it does seem that the results of the experiment are worthy of thought in considering the rationale for the use of procaine intravenously as an antagonist to histamine.

Summary

Procaine intravenously has been used with varying success for several years to combat various allergic phenomena. Past experimental studies have indicated that it acts as an antagonist to histamine. In the present experiments, it failed to affect the release of histamine by blood cells, and it failed locally to protect the skin from histamine-induced wheals. Locally, it does seem to give some protection from inflammation induced by a primary irritant. It diminishes the histamine-induced contractions of smooth muscle, but only in concentrations phenomenally greater than therapeutic blood levels either of itself or of its hydrolytic products. Significantly, it potentiates an antigen-antibody reaction locally induced in the rabbit's skin, and intravenously, it potentiates the toxicity of histamine to respiratory function in puppies.

The interpretation of these experiments is vague in relation to clinical use of procaine intravenously against allergic phenomena. However, its therapeutic efficacy seems to remain an empirical observation not well supported by experimental pharmacological observations.

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EFFECTS OF ACETYL CHOLINE ON LACTATION IN GUINEA PIGS, RATS AND HUMANS*

FREDERICK E. EMERY, CLARENCE E. GOSSETT, WILLIAM C. YOUNG, AND EVA DODGE†

The possibility that milk secretion may be modified by acetyl-choline led us to undertake experiments on the mammary glands, pregnancy and lactation in animals in which the amount of acetyl-choline was increased by injecting prostigmin.[1] We have approached this problem in two ways; (a) by giving estrogens[2] in constant amounts per week followed by prostigmin, and (b) by injections of prostigmin alone.

Table I shows data on four groups of guinea pigs. Group I consists of 25 oophorectomized animals given theelin in saline subcutaneously in amounts of 0.1 mg. daily. After two weeks, they were considered to be in a steady state in relation to estrogens. They were then tested for milk secretion by manual manipulation, and .05 mg. of prostigmin was injected. These procedures were repeated on the following day. On the third and fourth days they were tested for milk, but prostigmin was not given. The estrogens given in this way induced milk secretion in less than one-fourth of the guinea pigs. After prostigmin, milk was present in some of those not previously lactating. As will be seen in the table that 11% began to lactate, 86% showed no change, and 3% milk secretion stopped following injections of prostigmin.

Male guinea pigs injected with 1.0 mg. of theelin twice a week were given prostigmin as stated above. No effect on lactation was observed (Table I).

Pregnant guinea pigs were given 0.05 mg. of prostigmin daily for two consecutive days, and this was repeated each week during a period of 8 months. They were observed for milk secretion before prostigmin was given and on the second and

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[1]Prostigmine was kindly supplied by Dr. E. L. Sevringhaus, Hoffmann-La Roche, Inc.

[2]The estrogens in the form of theelin was kindly supplied by Dr. Oliver Kamm, Parke, Davis and Company.

TABLE 1

Milk secretion in guinea-pigs after prostigmin showing the number of animals followed by per cent.

Number of pigs	Kind	Number tests	Milk Secretion began	Milk Secretion no change	Milk Secretion stopped
25.....	Females	70	8–11.4%	60–85.7%	2–2.9%
36.....	Males	81	3– 3.7%	73–90.1%	5–6.2%
21.....	Pregnant	154	10– 6.5%	133–86.4%	11–7.1%
61.....	Controls	66	3– 4.5%	63–95.5%	None

third days following. It is well known that gestation inhibits lactation (Van Dyke, 1939) and many of these guinea pigs were not lactating even in late pregnancy. Lactation in these guinea pigs was not altered by prostigmin (Table I).

As shown in the table, milk secretion was obtained in three of 61 oophorectomized control guinea pigs given prostigmin only. In these cases, the milk secretion may have been due to loss of estrogens following oophorectomy (Nelson, 1937).

In addition to the milk secretion mentioned above, the twenty-one pregnant guinea pigs were observed for the number and care of their young. During a period of 8 months all but one of them gave birth once and several of them produced a second litter during the time they were on prostigmin. Lactation was adequate for rapid growth and 44% of the known fetuses lived. This compares with 48% of the living fetuses obtained in the saline injected controls.

Similar experiments were conducted in breeding rats. Prostigmin was given subcutaneously, and the amount was increased gradually so that after several days 0.1 mg. could be tolerated. This was continued daily for 90 days. There were 20 rats in this group and 70% gave birth to living young. The mean litter size was 5.75 rats. This compares with 90 and 5.1 for the per cent giving birth and litter size respectively in 20 control rats injected daily with 1 cc. of saline. The young from prostigmin injected mothers grew normally and looked well.

We have continued these studies in a series of 18 women in the last two weeks of pregnancy. One milligram of prostigmin was injected subcutaneously each four hours until signs of muscle twitching and restlessness were noted. The total dosages ranged from 3 to 60 mgm. Three of these patients delivered

elsewhere and their records are not available. One patient gave birth to a stillborn. The remaining fourteen patients delivered in the University Hospital. Three of the babies started with formula only, one started with breast and formula and the remaining ten were entirely breast fed. All babies were doing well at the time they were discharged.

Summary

From these studies it is evident that acetyl-choline induced by prostigmin has no noticeable effect on lactation in guinea pigs, rats and humans. The mammary glands developed and milk secretion occurred in advanced pregnancy even though prostigmin was being given. Growth of the young was not affected. Acetyl-choline appears to be of no significant value in lactation.

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PROTECTION OF IRRADIATED RATS BY PARABIOSIS*

JOHN C. FINERTY, ROBERT BINHAMMER AND MARTIN SCHNEIDER

It has been shown recently by Brecher and Cronkite (1951) that post-irradiation parabiosis is followed by an increase in survival-time of rats which had been exposed to a lethal dose of X-radiation. In their experiments, 14 rats were irradiated with 700 r and placed in parabiosis with intact non-irradiated littermates within a few hours. After 28 days the remaining 6 pairs were separated. Five of the irradiated rats were alive and gaining weight 3½ months later. Pre-irradiation parabiosis has also been successful in reduction of lethal effects of X-rays (Woenckhaus, 1930; Barnes and Furth, 1943; Van Dyke and Huff, 1949; Edwards and Sommers, 1952).

The present experiments are an attempt to confirm and extend the observations of Brecher and Cronkite (1951) and to study the role of the adrenal glands and spleen in resistance to radiation sickness.

Materials and Methods

X-radiation was delivered with the following factors: 200 KV, 30 ma, 0.25 Cu, 1.0 A1, HVL 0.62 Cu, 50 cm. STD, 20 x 20 cm. field, intensity 78 r/min. The rats were placed in cardboard boxes 20 x 6 x 5 cm. in size. Two boxes, each containing one rat, were centered, side by side, in the 20 x 20 cm. field of exposure, and total body irradiation was directed to the dorsal surfaces of the rats.

Parabiosis was performed under nembutal anesthesia according to the method of Bunster and Meyer (1933) using littermate rats of approximately equal weight.

Twenty-three female rats of the Holtzman strain, weighing about 115 grams, were irradiated with 827 r (air). Within a few hours 8 of them were placed in parabiosis with intact, non-irradiated, littermate partners; 10 were joined to littermate

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rats which were adrenalectomized[1] at the time of parabiosis; and 5 were joined to littermates which were splenectomized at that time. After 30 days the surviving pairs were separated surgically and observed for an additional 30 day period.

Thirty similar single rats were irradiated with 710 r, 827 r or 1240 r and served as controls.

Results

The results are presented in Table I. Sixty days after irradiation and pairing, 7 of 8, or 87 per cent of the paired rats which had received 827 r were alive, whereas only 1 of 17, or 6 per cent of the unpaired rats which had received the same dose survived. Those pairs in which one partner was irradiated and the other adrenalectomized had a 50 per cent survival. When the adrenalectomized-irradiated pairs were separated, 4 of the 5 adrenalectomized partners died of adrenal insufficiency, but the irradiated partners survived the experimental period. Splenectomy of the rat placed in parabiosis with an irradiated partner did not reduce the survival, since 5 of 5 such pairs persisted in

TABLE 1

Treatment		No. of pairs	No. alive at 30 days	Survival of parabionts 30 days after separation	
Left partner	Right partner			Left partner	Right partner
Irradiated 827 r	Normal	8	7 (87%)	7	7
Irradiated 827 r	Adrenal-ectomized	10	5 (50%)	5	1
Irradiated 827 r	Sple-nectomized	5	5 (100%)	5	5

Single intact controls	No of rats	No. alive at 30 days	No. alive at 60 days
Irradiated 710 r	7	6 (86%)	6 (86%)
Irradiated 827 r	17	3 (18%)	1 (6%)
Irradiated 1240 r	6	0 (0%)	0 (0%)

[1]Adrenalectomized rats were injected with 0.2 ml. lipoadrenal cortical extract (Upjohn) at the time of operation to help withstand the trauma associated with the first few days of parabiosis.

good condition throughout the period of pairing and all of the 10 rats lived through the additional 30 day observation period.

Irradiation of single rats with 1240 r resulted in death of all 6 rats within 5 days, whereas at 710 r, 6 of the 7 rats survived.

Discussion

At least 3 different mechanisms of death due to irradiation must be considered in relation to dosage and survival time (Cronkite and Brecher, 1952). These are the hyperacute syndrome at doses of over 12,000 r, which is accompanied by neuromuscular symptoms and leads to death in a few hours; the acute intestinal injury at 1,200–12,000 r (Quastler, *et al.*, 1951, in mice) which leads to death in 3½ to 5 days; and the bone marrow injury at lower doses with death at approximately 11 days.

In these experiments, the deaths are probably of the third category, primarily as a result of bone marrow damage, except for those control rats which received 1,240 r and died within 5 days.

Protection of the irradiated rats in this experiment is similar to that obtained by Brecher and Cronkite (1951), and confirms their results. The better percentage of survival in the present work probably indicates a slightly lower biological effectiveness of the dose of X-radiation, as is suggested by the survival of 1 of 17 controls subjected to 827 r (air), whereas the previous workers had no survivors of 104 controls, irradiated with 700 r (air). Differences in method as well as in biological effectiveness of irradiation may also contribute to difference in results, such as equal exposure to dorsal and ventral surfaces in the earlier experiments, and only dorsal exposure in the present work.

The absence of adrenal glands in the non-irradiated partner does not completely vitiate protection by post-irradiation parabiosis, since 50 per cent of such pairs survived. In such an experimental procedure each of the partners exerts a reciprocal protection in that either alone would probably not survive. Completely adrenalectomized rats usually die within 10–14 days after operation, but may be protected by parabiosis with an intact partner (Li and P'An, 1940; Copp and Finerty,

1950). Following 30 days of parabiotic existence, separation of the 5 remaining pairs showed that the protection from irradiation was complete, but that the protection from adrenal insufficiency lasted only during parabiotic attachment. Survival of 1 of the 5 adrenalectomized rats after separation was due to regeneration of adrenal cortical tissue.

All of the irradiated-splenectomized pairs survived in good condition during the period of parabiosis and the succeeding period of separation. These pairs differed only in that they seemed to be less affected by the irradiation and were in better health during the course of the experiment than other pairs. This impression has also been obtained by Brecher and Cronkite (1952) with unpaired, irradiated, splenectomized animals.

Some of the deaths of pairs in which one partner had been irradiated should not be attributed to the results of irradiation. Three of the pairs suffered death of one or both partners following the typical signs of parabiosis intoxication, a condition which commonly occurs in parabiotic rats (Finerty and Panos, 1951).

The irradiated rats in parabiosis showed fewer signs of radiation sickness than similarly treated single rats, although some hair loss was observed during the third week following exposure. Epilation occurred only in the irradiated rat, which does not support the observations of Van Dyke and Huff (1949) who found that both partners underwent epilation. Their pairs were placed in parabiosis, then irradiated while one partner was shielded. The unprotected partner received 900 r and the shielded one approximately 3 per cent or 27 r. A characteristic finding in the present experiments and in those of Brecher and Cronkite (1951) is the reduced weight of the irradiated partner which seems to persist even though the rat otherwise appears normal.

Summary

Female rats, weighing about 115 grams, were exposed to a lethal dose of X-radiation and placed in parabiosis with littermates within a few hours. Seven of 8 which were paired with intact non-irradiated partners survived for 30 days in parabiosis and at least an additional 30 day period after surgical

separation. Five of 10 which were paired with adrenalectomized rats survived during the same periods, but 4 of the 5 non-irradiated, adrenalectomized rats died shortly after surgical separation. All of the 5 rats joined to splenectomized partners survived in good condition.

These results support the conclusions of Brecher and Cronkite (1951) that parabiosis effectively protects rats from the lethal effects of X-radiation. Splenectomy of the non-irradiated partner does not reduce its protective ability, but a lower percentage of survival was obtained when that partner was adrenalectomized.

Grateful acknowledgment is made to Doctors R. C. Wybourn, Elmer Heimbigner and Roberto Calderon of the Department of Radiology for their assistance in this project.

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EFFECT OF FLUORIDE ON TUMOR GROWTH

JOHN C. FINERTY AND JAMES D. GRACE*

Fluorides are considered to be potent inhibitors of certain enzyme systems (Borei, 1945) and have been shown to exert a protective action against the toxicity of arsenic, which is known to be one causative agent for the production of cancerous growths (Arguello *et al.*, 1938). Arsenic trioxide poisoning has been prevented by the addition of potassium fluoride to drinking water (Finerty and Grace, 1945). Some concern has been shown about the possibility of increased tumor growth or susceptibility because of the increasing use of fluorides in public water supplies as a preventive measure for dental caries. The present experiment was conducted to determine whether any effect upon the growth of a transplanted sarcoma is exerted by a high level of fluoride in the drinking water.

Material and Methods

Twenty 30-day-old male rats of the Holtzman strain were injected subcutaneously along the abdominal wall on each side with equal amounts of a finely divided suspension of the Walker rat sarcoma 319. Twenty-five days later all rats showed tumor development on one or both sides, ranging from large lobulated masses to small, barely palpable lumps. The rats were arbitrarily separated into 4 groups, so that each group contained rats with similar tumor development to every other group. Group I was given .017% potassium fluoride (KF) in distilled water as its sole water supply; Group II received only .033% KF; Group III received only .05% KF; and Group IV received only distilled water. All groups were kept in adjacent cages under identical environmental conditions, and received the same diet consisting of Purina laboratory chow, *ad libitum*. Daily observation of the rats was continued throughout a period of 100 days.

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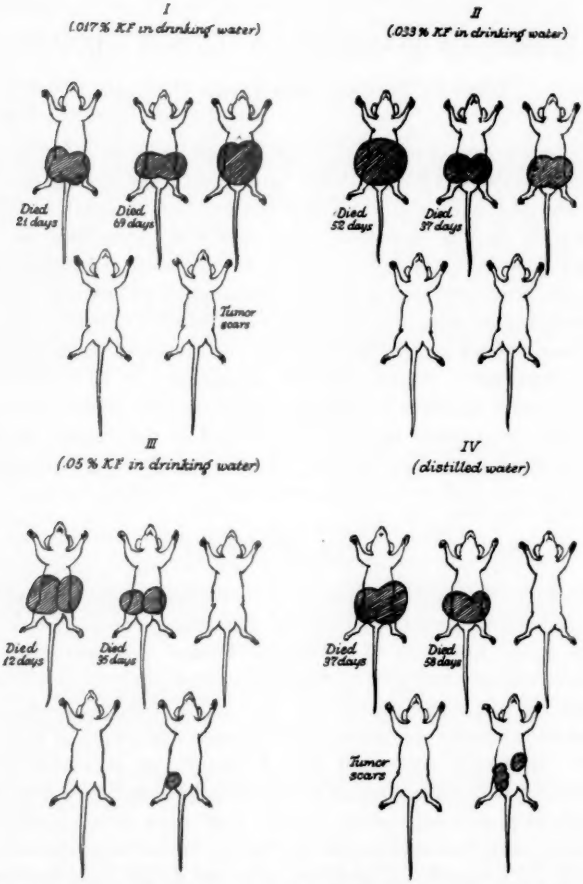

FIG. 1. The approximate size and shape of the tumors are illustrated in the rats of each of the four experimental groups at the termination of the experiment (100 days after beginning treatment) or at time of death of the rat.

Results and Discussion

The results are illustrated in Figure 1. The approximate size and shape of the tumors are shown at the time of death

of the rat, or at the termination of the experiment, 100 days following beginning of treatment. All of the rats which died during the course of the experiment had large lobulated, ulcerated tumors which presumably were the chief cause of death. Comparison of the groups shows that no significant difference exists between groups either in number of survivors, size of the tumors, or in number of complete tumor regressions. The only conclusion that seems possible is that under the conditions of this experiment KF treatment seems to have no effect on the progress of growth of the Walker rat sarcoma 319. The number of complete regressions appears high, but they undoubtedly occurred in those rats of each group which had only small tumors at the onset of treatment. Possibly the use of distilled water may have influenced this percentage by omission of some essential nutritional requirement, since the incidence of regression in rats from which the sarcoma was taken was lower. Further experiments are in progress to determine whether the use of distilled water modifies tumor growth.

Summary

Rats which had received implants of the Walker rat sarcoma 319 were given various amounts of potassium fluoride in their drinking water. There was no inhibition nor stimulation of tumor growth under the influence of fluoride.

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THE PROTECTIVE EFFECT OF CHEMOTHERAPEUTIC AGENTS ON A SPECIFIC IMMUNE STATUS OF X-RADIATED MICE. I. A PRELIMINARY STUDY*

JOHN D. FULTON, ANNA C. ARNOLD, MASIL B. DANFORD AND ROLAND B. MITCHELL

Introduction

Adrenal cortical extract has been shown to cause an enhancement of circulating antibodies (Mitchell *et al.*, 1950, 1951). Previous work on this project has further indicated that antibody titers in animals exposed to high dosages of whole body x-radiation can be maintained at a high level by the use of adrenal cortical extract (Mitchell *et al.*, 1950, 1951). Straube *et al.* (1949) have shown, however, that the administration of adrenal cortical steroids after x-radiation does not alter the mortality of x-radiated animals.

Limperos and Mosher (1950), Patt *et al.* (1949), Chapman and Cronkite (1950), and Cronkite *et al.* (1950) have shown that various sulfhydryl-containing compounds, such as thiourea, cysteine, and glutathione, exert a protective effect on animals exposed to whole body x-radiation when they are injected into animals prior to x-radiation.

Cronkite *et al.* (1950) have shown that lymphocytes and granulocytes were apparently absent from peripheral blood in untreated x-radiated animals but present in small numbers in animals treated with glutathione prior to x-radiation. It is a well-known fact that leukocytes are a first-line defensive agent against bacterial invasion and any treatment, such as x-radiation, which destroys the leukocytes considerably weakens the organism's ability to withstand bacterial invasion either from the normal gut flora or from other sources.

Miller *et al.* (1950) have shown that the survival time of x-radiated animals can be increased by the use of antibiotics. They attribute this increase in survival time to a control of the bacteremias caused by an invasion of microflora from the gut.

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This report gives the results obtained in a series of experiments designed to determine whether or not a specific immunity in x-radiated animals can be protected by treatment with certain substances that have previously been shown to exert some protective effect on animals exposed to x-radiation.

Experimental Procedure

Animals

The animals used throughout this work were male albino mice, CF-1 strain, weighing 22 to 25 grams.

Immunization

Each of the animals, except where otherwise noted, was immunized 6 days (144 hours) prior to x-radiation or other treatment with a 0.5 ml. intraperitoneal injection of *Salmonella typhimurium* antigen. This dose of antigen contained 750,000,000 heat-killed organisms suspended in normal saline.

Infective Dose

The infective dose was administered as an intraperitoneal injection of 0.5 ml. of a saline suspension of live virulent *S. typhimurium* organisms. This dose contained 750,000 organisms and was prepared as follows:

1. A tube of brain-heart infusion broth was inoculated with *S. typhimurium* and incubated for 18 hours at 37° C.
2. One-tenth ml. of the 18-hour culture was added to 9.9 ml. of brain-heart infusion broth and was incubated for 5 hours at 37° C.
3. The inoculum was prepared by diluting 1 part of the 5-hour culture with 249 parts of sterile saline.

Radiation

X-radiation was administered at the rate of 55 r/minute. The radiation factors were as follows:

260 kv.—18 ma.

Filtration of 1.0 mm. aluminum + 0.25 mm. copper.

Filament to target distance (center of animal)—70 cm.

The animals were exposed to whole body x-radiation in a box containing 25 plywood cells, each 2 x 2 x 2 inches. The

walls of the chambers were ¼-inch plywood. The top and bottom of the exposure box were constructed with ¼-inch Plexiglass. The outside dimensions of the box were 11½ x 11½ x 2½ inches. Each mouse was placed in an individual cell to prevent any huddling or piling up. Variation in x-radiation intensity in the field was no greater than 5 per cent.

Thiourea[1]

Thiourea was administered as an intraperitoneal injection of 0.5 ml. of a saline solution containing 80 mg. of thiourea per milliliter. The thiourea solution was sterilized by filtration through a Seitz filter and administered 5 minutes prior to x-radiation.

Adrenal Cortical Extract[2]

The adrenal cortical extract (ACE) was administered as a subcutaneous injection in the back. Each animal receiving this substance received a dose of 5 rat units or 0.125 mg. of 17-hydroxycorticosterone. This quantity was contained in 0.125 ml. of cottonseed oil and was administered 1 hour prior to x-radiation. In those experiments in which ACE was used, the animals that did not receive ACE received a control subcutaneous injection of 0.125 ml. of sterile cottonseed oil (CSO).[3]

Glutathione

The glutathione dosage used in this work was identical with that used by Chapman and Cronkite (1950). They have shown that glutathione increases the survival time of x-radiated animals when it is injected prior to irradiation. They have further shown that the amount of protection conferred is directly proportional to the quantity of glutathione administered, and that it is much more effective at higher x-radiation dosages than at lower. The dose of glutathione administered was 4 mg. per gram of body weight and was contained in a 1 ml. dose injected subcutaneously (in the back) 30 minutes prior to x-radiation. The glutathione was in aqueous solution and the pH was adjusted to 6.5 with 10 per cent sodium hydroxide. The solution was sterilized by filtration through a Seitz filter.

[1]Pfanstiehl Chemical Co.
[2]Lipo Adrenal Cortex—Upjohn Co.
[3]The cottonseed oil was kindly supplied by the Upjohn Company, Kalamazoo, Michigan.

Reticulose[4]

Berry and Mitchell (1951) have shown that injection of Reticulose, a lipoprotein-nucleic acid complex, into mice prior to infection increased the resistance and prolonged the survival time of mice infected with *S. typhimurium* organisms. It was thought possible that this substance might protect and/or stimulate the reticulo-endothelial system during and after x-radiation. A 0.5 ml. dose of Reticulose was injected subcutaneously (into the back of the animal) 30 minutes prior to x-radiation.

Chloromycetin[5]

Antibiotic sensitivity tests indicated that the strain of *S. typhimurium* used as the infective organism was quite sensitive to chloromycetin. Each animal treated with chloromycetin received 1.25 mg. of chloromycetin twice daily, throughout the course of the experiment, as a subcutaneous injection in the back.

Results and Discussion

Experiment I

The initial experiment was designed to determine the effect of adrenal cortical extract (ACE) and thiourea, administered singly and in combination prior to x-radiation, on the survival of mice immunized to *S. typhimurium*, then x-radiated, and subsequently infected with virulent *S. typhimurium* organisms.

The radiation dosage used in this experiment was 450 r whole body x-radiation and was administered as previously described.

The experiment was designed as shown in Table I.

Each of the animal groups employed in this experiment received immunization, 450 r whole body x-radiation, and infection, with the exception of the "immunization control" group, which received immunization and infection but was not exposed to x-radiation. This group (immunization control) was used to determine the effectiveness of the immunization against the subsequent infection with *S. typhimurium*.

[4]Chemico Laboratories.

[5]Chloromycetin-Parenteral was kindly supplied by Dr. H. E. Carnes of Parke-Davis & Co.

TABLE 1

Schema of Experiment I—450 r Whole Body X-radiation

Group	a Thiourea	b ACE	No. of animals
(1)	O	O	24
a	+	O	24
b	O	+	24
ab	+	+	24
Immunization control	O	O	24

+—presence of factor.
O—absence of factor.

In addition to immunization, radiation, and infection, group *a* received thiourea administered 5 minutes prior to x-radiation, group *b* received adrenal cortical extract 1 hour pre-radiation, and group *ab* received both thiourea (5 minutes pre-radiation) and adrenal cortical extract (1 hour pre-radiation).

All groups received an infective dose of Virulent *S. typhimurium* organisms approximately 24 hours after x-radiation.

The animals were observed throughout the duration of the experiment and the time of death for each animal was recorded. The deaths were recorded hourly throughout the day until 2100. Animals that died between 2100 and 0800 were recorded as dying at 0800 the following day. The experiment was terminated at 309 hours post-infection. The results are shown in Figure I, in which the percentage of survival is plotted against

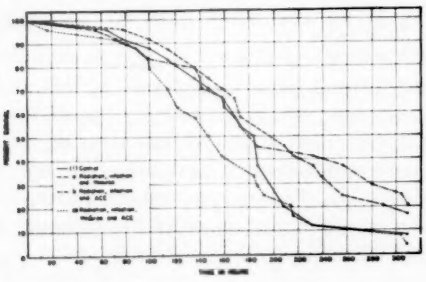

FIG. I. Survival Time of X-Radiated-Infected Animals Treated with Thiourea and ACE.

time. The actual time of death in hours post-infection is shown in Table II.

TABLE 2

Time to Death (Hours Post-infection) in Experiment I

Animal No.	(1) Control	a Thiourea	b ACE	ab Thiourea and ACE
1	64	77	55	16
2	77	99	72	71
3	99	114	88	88
4	114	136	98	98
5	136	136	136	99
6	136	147	140	114
7	147	158	142	114
8	158	168	158	122
9	160	173	168	122
10	168	173	168	136
11	173	184	173	147
12	184	184	187	147
13	187	208	187	158
14	187	216	232	158
15	187	232	256	184
16	208	238	280	184
17	208	256	280	187
18	208	256	304	192
19	208	289	309	213
20	232	309	...	216
21	232	...	...	232
22	309	...	...	304
23	...	...	...	309
24	...	...	...	...

NOTE: The first animal that died was given the number 1, the second animal that died was numbered 2, etc.

As can be observed in Figure I little, if any, protection was conferred to the animals by the tratments employed. Up to 190 hours the animals which received treatment with thiourea (group *a*) and those animals which received ACE (group *b*) survived no longer than did those animals which received no treatment. However, after 190 hours these treated groups tended to die less rapidly than did the nontreated group. It is also apparent from the graph that those animals receiving the combination treatment of thiourea and ACE (group *ab*) died more rapidly than did the nontreated animals (group (1)).

It can be concluded from this experiment that neither adrenal cortical extract nor thiourea increased the survival time of immunized, x-radiated, and infected animals. An important

and perhaps significant point for future consideration is the apparently adverse effect of the combination treatment of thiourea and adrenal cortical extract. The animals receiving this treatment died more rapidly than did those animals receiving immunization, radiation, and infection alone. It may be that some antagonistic effect occurs between the two substances when they are administered in combination. The significance of these results cannot be satisfactorily evaluated at this time and will require considerably more study and experimental work.

Statistical Analysis

For this experiment, the treatments were:

(1) Control.
(a) Thiourea.
(b) ACE.
(ab) Thiourea + ACE.

To account for the fact that the experiment was by necessity terminated at an arbitrary hour (309 hours in this experiment), it is felt that the reciprocal of survival time is a better variable to study. In using reciprocals we can set 1/309 equal to zero, which has the effect of assuming the animal died at a much later date of natural causes. If we had used the time of survival in hours or the logarithms of time of survival, we might either assume all the animals not dead at the end of the experiment as having died at the 309th hour or use some procedure to estimate the statistics from a truncated distribution. It is felt that the animals have developed immunity because of the treatments so that reciprocals should be studied.

The results for this experiment are shown below. All means quoted have been converted back to hours. It is important to note, however, that these means are *not* the same as the average of the original survival time in hours.

The A main effect is a comparison of treatment (a) and ($\bar{a}$) [absence of (a)] over treatments (b) and ($\bar{b}$) [absence of (b)]. Similarly the B main effect is a comparison of treatment (b) and ($\bar{b}$) over (a) and not ($\bar{a}$).

From the analysis of variance (Table III) we see that neither the A (thiourea) nor the B (ACE) main effects are significant.

TABLE 3

Analysis of Variance in Experiment I

Source	d.f.	M.S.	F	P
A	1	.00004070	.876	.20
B	1	.00007857	1.691	.198
AB	1	.00014095	3.033	.090
Error	84	.00004647		
Total	87			

The AB interaction term has a fairly low probability (chance of 1 out of 11). The reason for this can be seen from an inspection of the means (Table IV). The mean for treatment (a) is smaller in the presence of (b) than in the absence of (b). However, the reverse is true for ($\bar{a}$). (Note the arrows in the table.)

TABLE 4

Mean

(In hours; reciprocal of reciprocal means)

	b	$\bar{b}$
a	102.8 ↓	→ 188.3
$\bar{a}$	171.2	← 154.3 ↑

Conclusion. Neither ACE nor thiourea significantly increased survival time; however, it is important to notice that the two treatments in combination had a deleterious effect. This effect may have been real, but in this experiment it was not significant. A study of the histograms in Figure II, charts 1–4 (Experiment I) will be helpful.

Experiment II

The second experiment in the series was designed to determine if glutathione, ACE, Reticulose, and cottonseed oil administered prior to x-radiation would prolong the survival time of mice immunized, x-radiated, and subsequently infected with virulent *S. typhimurium* organisms.

The experiment was made of four series of five groups, each group having 20 animals. Each series received the same test

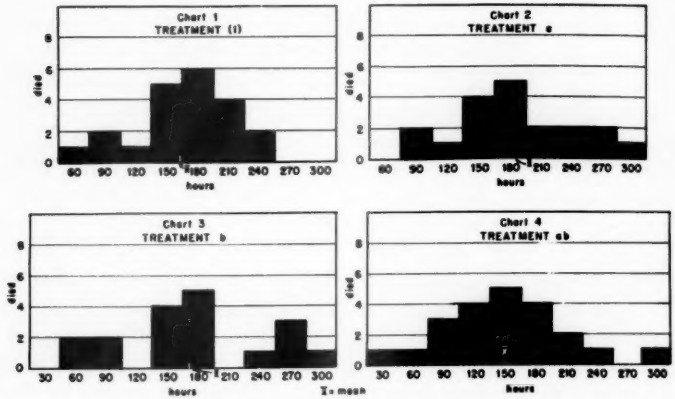

FIG. II. Histograms for Experiment I.

$\bar{x}$ = mean

substances but a different dose of whole body x-radiation. The x-radiation doses employed were as follows:

Series I— 250 r—a_1
Series II— 500 r—a_2
Series III— 750 r—a_3
Series IV—1000 r—a_4

Each group of animals in all four series received immunization, x-radiation, and infection.

The animals were immunized, treated with the test substances, and infected as previously described. The animals used in this experiment received the infective dose of *S. typhimurium* 24 hours post-radiation.

The standard schema using one of the doses of x-radiation (Series I) is shown in Table V. The schemata for the remaining series are identical when the proper x-radiation value is substituted in the radiation column on the schema.

Inasmuch as cottonseed oil was the vehicle in which the ACE was carried, a group receiving cottonseed oil (CSO) alone was included in the experiment as a control measure. The animals in this group received 0.125 ml. of CSO injected subcutaneously in the back.

TABLE 5

Schema for Series 1—250 r Whole Body X-radiation

Group	a_1 250 r x-radiation	b CSO	c ACE	d Glutathione	e Reticulose	No. of animals
a	+	0	0	0	0	20
ab	+	+	0	0	0	20
ac	+	0	+	0	0	20
ad	+	0	0	+	0	20
ae	+	0	0	0	+	20

+—presence of factor.
0—absence of factor.

Times of death were recorded at 12-hour intervals throughout the duration of the experiment. When the time of death (in hours) was plotted against percentage of survival, there was no apparent difference in the survival rate of x-radiated, infected animals (in all four radiation series) and comparable groups of animals treated with CSO, ACE, and Reticulose. Inasmuch as there was no apparent protection conferred on the animals by treatment prior to x-radiation with these substances, no further evaluation or discussion of the results obtained with these substances will be included.

In each x-radiation dosage series, however, the glutathione-treated animals did not die as rapidly as did the nontreated animals. In the 250 r series (Series I) this holds true only up to 200 hours, after which they died more rapidly than did the nontreated animals.

The results obtained in all four series are shown graphically in Figure III. The actual time of death in 12-hour intervals post-infection is shown in Table VI.

Table VII shows the calculated times (in hours) for the glutathione-treated and nontreated animals to reach survival levels of 75, 50, 25 and 0 per cent.

A study of Table VII reveals that the 750 r glutathione-treated animals give a similar survival picture, at all survival levels to the 500 r nontreated animals. Apparently at this x-radiation level (750 r) the glutathione conferred some protection to the immune systems of the animals.

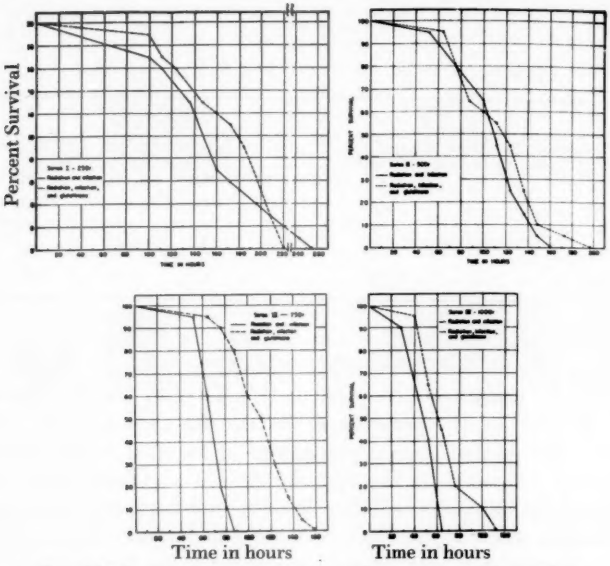

FIG. III. Survival Time of Animals Treated with Glutathione and X-Radiated with Various Dosages of X-Radiation.

TABLE 6

Time to Death (12-Hour Intervals—Post-infection) in Experiment II

Animal No.	250 r		500 r		750 r		1,000 r	
	Control	Glutathione-treated	Control	Glutathione-treated	Control	Glutathione-treated	Control	Glutathione-treated
1	100	100	52	64	52	64	40	40
2	100	112	76	88	64	76	40	52
3	100	112	76	88	64	88	52	52
4	112	124	76	88	64	88	52	52
5	136	148	100	88	64	100	52	52
6	136	148	100	88	64	100	52	52
7	136	148	100	88	64	100	52	52
8	148	160	112	100	64	100	52	64
9	148	172	112	112	76	112	52	64
10	148	184	112	124	76	112	52	64
11	160	184	112	124	76	124	52	64
12	160	196	124	136	76	124	52	76
13	160	196	124	136	76	124	64	76
14	172	208	124	136	76	124	64	76
15	196	208	124	136	76	136	64	76
16	196	208	136	148	76	136	64	76
17	208	208	136	148	88	136	64	100
18	244	220	148	148	88	148	64	100
19	244	220	148	172	88	148	64	112
20	256	220	160	196	88	160	64	112

Statistical Analysis

For the second experiment, four levels of x-radiation (250 r, 500 r, 750 r, and 1,000 r) were administered. One level of glutathione was given. It was desired to see at what level of x-radiation glutathione affords the greatest protection to immune, x-radiated, and infected animals. The treatments were:

(a) X-radiation + infection
(d) Glutathione + infection
(ad) X-radiation + glutathione + infection

The subscripts on the a's shall designate the level of x-radiation, *e.g.*, $a_1 = 250$ r, $a_2 = 500$ r, $a_3 = 750$ r, $a_4 = 1{,}000$ r.

The analysis was run simultaneously for all four levels.

The (a) versus (ad) comparison (Table VIII) indicates that glutathione increased survival time significantly. A look at the means (Table IX) gives a clearer picture.

TABLE 7

Survival Levels of Glutathione-Treated and Untreated X-radiated Animals

Series	Glutathione	Time in hours to survival level of— 75%	50%	25%	0%
I (1,000 r)	O	44	50	57	64
	+	48	60	73	112
II (750 r)	O	60	66	72	84
	+	90	112	126	160
III (500 r)	O	92	110	124	160
	+	84	118	136	196
IV (250 r)	O	128	148	190	260
	+	140	178	202	220

+—Presence of glutathione.
O—Absence of glutathione.

TABLE 8

Analysis of Variance in Experiment II

Source	d.f.	M.S.	F	P
Treatments	7	.00038319	51.923	<.001
a vs. ad	1	.00020039	27.153	<.001
$a_{(linear)}$	1	.00156464	212.011	<.001
$ad_{(linear)}$	1	.00077543	105.072	<.001
$a_{(quadratic)}$	1	.00001108	1.501	<.20
$ad_{(quadratic)}$	1	.00005427	7.354	<.01
Remainder	2	.00003824	5.182	<.01
Error	152	.00000738		

TABLE 9

Means
(In hours; reciprocals of the reciprocal means)

a_1	a_1d	a_2	a_2d	a_3	a_3d	a_4	a_4d
150.4	163.7	104.6	111.5	71.6	109.1	54.5	65.2

A "t" test shows that the difference between (a) and (ad) is significant only at the 750 r and 1,000 r levels, and that glutathione is apparently the most effective at the 750 r level.

The significance of (a_{linear}) shows that in the absence of glutathione, the effect of the different levels of x-radiation has a linear effect on survival. ($a_{\text{quadratic}}$) is not significant, which suggests that apparently there is no "falling-off effect" at the higher levels of x-radiation.

The significance of (ad_{linear}) and ($ad_{\text{quadratic}}$) are of interest. There is a definite linear trend as well as a falling-off of the effect on survival at the higher level. This is pointed out above when we saw that at the 750 r level, glutathione is more effective than at the 500 r and 1,000 r levels.

Conclusions. Glutathione definitely increased survival rates of immune infected animals at the 750 r and 1,000 r x-radiation levels. It was most effective at the 750 r level. The effect was curvilinear. X-radiation had a significant linear effect on survival time. The survival time decreased with increasing radiation. This is clearly indicated in Figure IV, charts 1–8.

Experiment III

It was apparent from the preceding experiment that glutathione increased the survival time of animals exposed to 750 r whole body x-radiation and subsequently infected with virulent *S. typhimurium* organisms. The glutathione served to either protect the animal's immune defensive systems from the effects of x-radiation or enhanced them after they had been impaired as a consequence of the x-radiation.

This series of experiments was designed to determine if the survival time of immune, x-radiated, and infected animals could be increased by pre-radiation treatment with glutathione and post-radiation treatment with an antibiotic to which the infective organism was susceptible. Sensitivity tests had shown

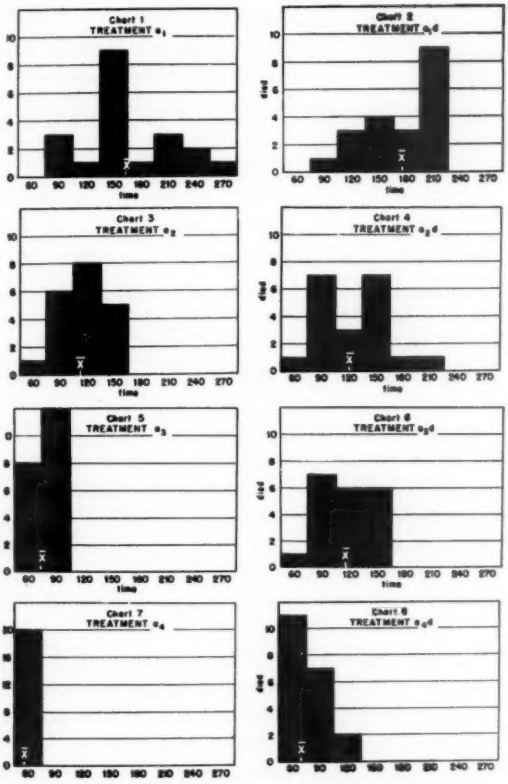

FIG. IV. Histograms for Experiment II.

$\bar{x}$ = mean

that the strain of *S. typhimurium* used as the infective agent was much more sensitive to chloromycetin than to the other common antibiotics.

By protecting and/or enhancing the immune defensive systems with glutathione prior to x-radiation and giving daily post-radiation doses of chloromycetin, it would be expected that the survival period of animals so treated would exceed that of animals which did not receive the treatment. The schema of this experiment is shown in Table X.

TABLE 10

Schema for Experiment III—750 r Whole Body X-radiation

Group	a Glutathione	b Chloromycetin	No. of animals
(1)	O	O	24
a	+	O	24
b	O	+	24
ab	+	+	23
Radiation control	O	O	24
Immunization control	O	O	19

+—Presence of factor.
O—Absence of factor.

Each group in the experiment, with the exception of the "radiation control" and the "immunization control" groups received immunization, x-radiation, and infection. The radiation control group received immunization and x-radiation but no infection or further treatment. This group served as a control group to determine the effect of x-radiation alone. The immunization control group received immunization and infection alone. This group was a control group to determine the effectiveness of the immunization against the subsequent infection.

The animals received the immunizing dose 6 days prior to x-radiation or other treatment. The radiation dosage used in this experiment was 750 r whole body x-radiation. X-radiation, immunization, infection, and treatment with glutathione and chloromycetin were accomplished as previously described. The initial dosage of chloromycetin (groups *b* and *ab*) was administered as a subcutaneous injection of 5 mg. 2 hours after x-radiation. The animals were infected 24 hours after x-radiation. All groups of animals except one (radiation control), received the infective dose. All of the groups received the immunizing dose 6 days prior to x-radiation or other treatment.

The animals were observed throughout the experiment and the hour of death in each case was recorded. The results are shown in Figure V in which the percentage of survival is plotted against time. The actual time of death in hours post-infection is shown in Table XI. The experiment was terminated after 188 hours.

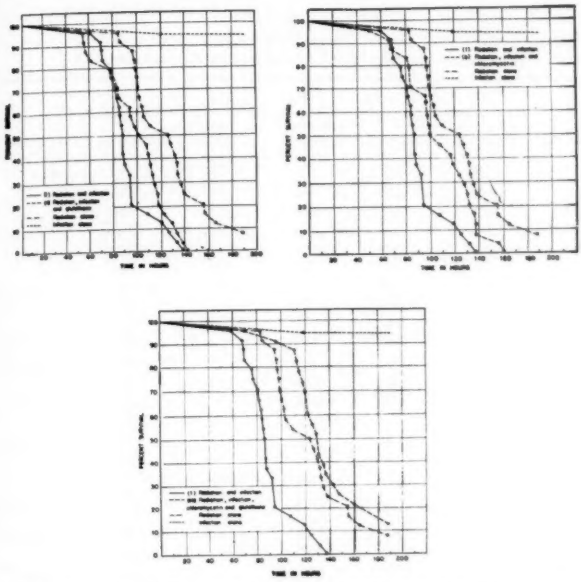

FIG. V. Survival Times of X-Radiated (750 r) Infected Animals Treated with Glutathione and Chloromycetin.

The results obtained in this experiment confirm the results obtained in the previous experiment; that is, glutathione increases the survival time of x-radiated infected animals. The increase in survival time in this experiment was not as great as that observed in the preceding experiment. The animals used in both experiments were from the same stock colony, and the glutathione dosage was increased to correspond to the increase in weight. The only basic difference between the two experiments was in the age of the animals. The animals used in the previous experiment were approximately 50 days old at the time of x-radiation, whereas the animals in this experiment were approximately 66 days of age. According to Abrams (1951) there would be little, if any, significant difference in x-radiation susceptibility between these two age groups.

Chloromycetin alone increases the survival time of x-radiated and infected animals. In this experiment the chloromycetin increased the survival time to approximately the same

TABLE 11

Time to Death
(Hours post-infection of animals in Experiment III)

Animal No.	(1) Radiation infection control	a Glutathione	b Chloro-mycetin	ab Glutathione and chloro-mycetin	Controls Radiation	Controls Infection
1	59	55	50	70	73	119
2	68	56	66	96	85	...
3	69	57	68	111	95	...
4	70	62	80	113	96	...
5	76	79	83	115	97	...
6	77	84	84	118	98	...
7	81	84	84	120	99	...
8	82	85	96	122	100	...
9	83	94	97	122	102	...
10	84	95	97	126	104	...
11	85	98	99	129	110	...
12	86	102	100	131	124	...
13	87	108	108	131	127	...
14	87	110	116	135	130	...
15	88	111	118	137	131	...
16	92	113	123	143	133	...
17	93	115	128	148	135	...
18	94	117	130	160	138	...
19	94	119	132	188	154	...
20	107	123	136	188	155	...
21	119	130	137	...	165	...
22	125	134	138	...	...	...
23	132	139	157	...	...	...
24	148	142	160	...	...	...

degree as did treatment with glutathione before x-radiation. This holds true up to 110 hours, after which time the chloromycetin apparently was superior to the glutathione. Chloromycetin or glutathione, when administered alone, did not give complete protection from the specific infection. When combined, however, the survival time of the treated animals was increased considerably over that of animals receiving x-radiation alone. As shown by an increase in the survival time, this treatment not only conferred complete protection to the animals from the specific infection, but also a degree of additional protection so that the x-radiated, treated, and infected animals survived for longer periods of time than did the animals which received x-radiation alone. This seems to indicate that pre-radiation treatment with glutathione and post-radiation treatment with chloromycetin protected the immune systems from the effects of x-radiation or enhanced them after they had been partially destroyed by the x-radiation.

Statistical Analysis

Experiment III is a factorial experiment with the indicated treatments:

(1) Control.
(a) Glutathione.
(b) Chloromycetin.
(ab) Glutathione + chloromycetin.

(R) Radiated but not infected.
(I) Infected but not radiated.

The x-radiation level used in this experiment was 750 r.

Only one animal died because of treatment (I). A "t" test of treatment (R) against treatment (1) indicates that very probably (probability $<.01$) radiated animals not infected survive longer than radiated-infected animals. From these two results it is obvious that animals infected only or radiated only survive longer than radiated-infected animals.

The analysis of the factorial part of the experiment (Table XII) shows that the A main effect (glutathione) definitely

TABLE 12

Analysis of Variance for Experiment III

Source	d.f.	M.S.	F	P
A	1	.00008496	8.813	$<.01$
B	1	.00016118	16.720	$<.001$
AB	1	.00003224	3.344	.076
Error	91	.00000964		

increases survival time. This was shown to be true in Experiment II. Also from this analysis we deduce that the B (chloromycetin) main effect also significantly increased survival time.

Why the AB interaction term failed to fall below the 5 per cent level is apparent from the following table of means (Table XIII). Treatment (a) affords greater protection in the presence of (b). Likewise, treatment ($\bar{a}$) has the same pattern. Similar statements could be made about treatment (b). It is true that treatment (ab) gives significantly greater protection than either (1), (a), or (b). However, this increase is not large enough to give rise to a significant AB interaction.

TABLE 13

Means
(In hours; reciprocals of reciprocal means)

a	b	$\bar{b}$	R
a	142.5	← 92.9	125.6
$\bar{a}$	99.4 ↑	← 87.0	....

Conclusions. The main effects A and B gave significantly greater protection as measured by survival time. It is interesting to note, however, that the AB interaction was not significant. For the interaction term to be significant, the (ab) treatment should give much greater protection than the additive effects of treatments (a) and (b). That is, treatment (a) would implement (b) rather than simply adding its effect to (b). To say that the AB interaction is not significant is *not* the same as saying that the treatment combination (ab) is not significantly better than treatment (1), (a), or (b). As a matter of fact, a "t" test of (ab) versus (1), and (ab) versus (a) shows that (ab) affords much greater protection than either (1 per cent level), while a test of (ab) versus (b) indicates that (ab) is considerably better than (b) (5 per cent level). These results are shown graphically in Figure VI, charts 1–5.

Figures II, IV, and VI show the number of animals that died at the indicated hours. A comparison of the histograms should include not only a study of the relative positions of the means but, also, a study of the spread or dispersion. It is important to note that the histograms are plots of the number of mice that died against the number of hours to death. However, the trend of the means is the same as for the reciprocals. The means on the figures will not, therefore, equal the means as quoted above in the analysis.

Summary

Male CF 1 mice were immunized with heat-killed *S. typhimurium* antigen. Six days after immunization the animals were divided into groups and given treatment with one or a combination of the chemotherapeutic agents employed. The animals treated with chloromycetin received daily doses

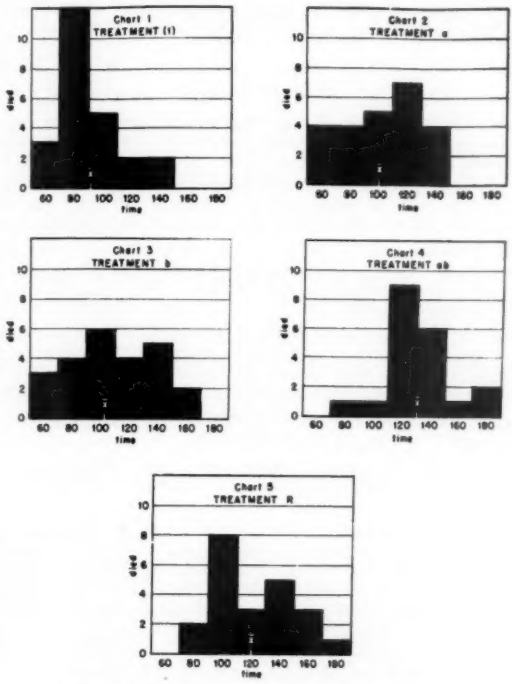

FIG. VI. Histograms for Experiment III.

$\bar{x}$ = mean

throughout the experiments. After treatment with the chemotherapeutic agents the animals were exposed to x-radiation and then infected with the virulent *S. typhimurium* organisms.

Under the conditions of the experiment it was observed that pre-radiation treatment with glutathione alone and post-radiation dosages of chloromycetin alone increased the survival time of immunized, x-radiated, and infected animals. The combination of pre-radiation glutathione and post-radiation chloromycetin increased the survival time considerably more than did either of the agents when administered alone. Administration of adrenal cortical extract, Reticulose, and thiourea, immediately

prior to x-radiation did not alter significantly the survival time of the infected experimental animals.

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INFANTILE DIARRHEA IN TEXAS*

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I. MAGNITUDE OF PROBLEM

Upon institution of a Child Health Program by The University of Texas, it was apparent that special attention must be given to the problem of infantile diarrhea. The importance of this matter is readily apparent from the data obtained from the Vital Statistics of the United States relative to the number of deaths included in the categories—diarrhea, enteritis and dysentery—for the first two years of life for Texas and the whole country. These are summarized in Figure 1, data for 1948 being the latest available. Although the trend since 1941 has been for a marked decrease in the total number of deaths in this category in the United States, in Texas there has been the reverse. Texas comprises about 5 per cent of the population of the United States. In 1941, about ten per cent of the diarrhea deaths in the country occurred in Texas infants, whereas, in 1948, twenty-five per cent of the diarrheal deaths in this age group were in Texas children. Over this eight year period (1941–1948), 15.7 per cent of all the deaths in the country attributed to diarrhea, enteritis and dysentery in the first two years of life occurred in Texas children.

Where one finds a large number of deaths from infantile diarrhea one is apt to find the deaths from other causes also to be high. Deaths from all causes in children under two years of age for United States and for Texas and New York have been ascertained and are presented in Table I. The number of deaths each year in these two states is in the same order of magnitude yet New York has approximately twice the population as Texas.

In order to ascertain the relative importance of diarrhea, enteritis and dysentery as a cause of death in children under two

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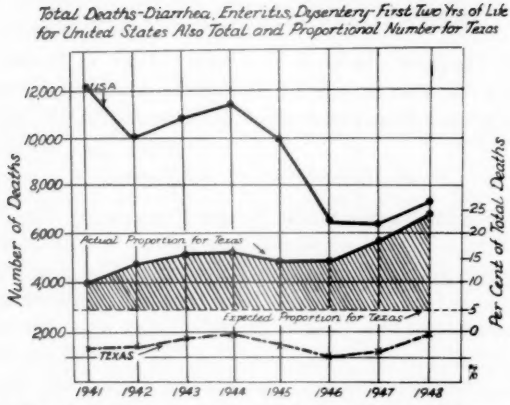

FIG. 1. Total deaths from diarrhea, enteritis and dysentery during first two years of life for the United States from 1941–1948, also the total and proportional number for Texas.

TABLE 1

Total deaths during first two years of life in Texas, New York and the United States from 1941 through 1948.

	1941	1942	1943	1944	1945	1946	1947	1948
Texas	8,600	8,636	9,334	9,349	8,438	8,295	8,757	10,013
New York....	7,451	8,350	8,724	8,089	7,947	8,845	9,552	8,892
U.S.A.	125,516	123,559	129,610	121,917	114,848	119,384	126,530	121,781

years of age, deaths resulting from various common disorders have been studied. These data are summarized in Figure 2. It is apparent that the most frequent causes of death in the first two years in the United States in the order named are: (1) prematurity, (2) congenital malformations, (3) pneumonia and influenza, then (4) the diarrhea, enteritis, dysentery category. Together these four categories include about 65 per cent or nearly ⅔ of the total deaths in this age group. In New York, prematurity is by far the greatest single cause of death in infants, congenital malformations next, then diarrhea. In Texas, on the other hand, after prematurity, the diarrheal disturbances far outrank the other causes. In 1948, during the first year of life, about 6.6 per cent of deaths due to prematurity in the

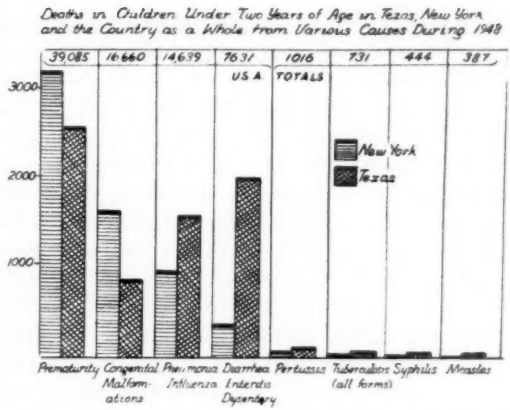

FIG. 2. Deaths in children under two years of age in Texas, New York and the country as a whole from various causes during 1948.

United States occurred in Texas, whereas 22.7 per cent of those due to diarrhea, enteritis and dysentery were in this state.

When one learns that diarrhea is an important cause of death in our infants, the question is immediately asked, "Why is this?" The greatest single contributory factor is racial. The exact proportion of Latin-American peoples in Texas cannot be determined from United States census figures; however, studies made by the Texas State Department of Health indicate that the deaths from diarrhea are greatest in the counties of the state with admittedly high Latin-American populations. Although additional evidence is desirable, none is known which would indicate that the death rate in the one-seventh of the Texas population classified as non-white (negro) is in excess by comparison.

In many states the greatest hazard of infantile diarrhea arises from the devastating outbreaks which occur during the neonatal period. In 1944, in New York 1.5 per cent of the deaths from diarrhea occurred in infants under one month while in Texas it was 1.0 per cent, hence, the proportion of deaths in the neonatal period is not significantly different in these two states. One can conclude from these and other data that during the first month of life excessively high death rates from this cause

do not occur among Texas infants. In other words, epidemic diarrhea of the new born is not responsible for the excessive deaths in Texas infants.

The handmaidens of high death rates in infants are ignorance, indigence, and indifference. The maid of honor among these is lack of knowledge. Hence, a Child Health Program under the auspices of a university directs its attentions to the solution of problems along the lines of education—teaching and research. In the ensuing sections of this communication a review is presented of the results of studies aimed at ascertaining possible etiologic factors. This has dwelt on the bacteriologic aspects of the problem and the influence of newer chemotherapeutic and antibiotic agents on enteric pathogens. The next portion concerns studies which deal with therapeutic measures. Basically, solution of the problem necessitates a clear understanding of the principles of therapy as they are presented to medical students, nurses and physicians, which in turn profoundly influences the education of the populace. Not only are young mothers and physicians concerned with this phase of the problem but also the general public, legislators and leaders in political and social life.

Summary

The disease category—diarrhea, enteritis and dysentery—is a major cause of death of infants in Texas. In the United States in 1948, about one-fourth of all the deaths which occurred in this category were in children of Texas, yet this state has only about one-twentieth of the population.

II. Causative Bacterial Agents in Infantile Diarrhea

A. Pathogens and possible pathogens in infants with diarrhea and in control subjects:

Shortly after the Child Health Program was instituted a laboratory for studying enteric organisms was established. Bacteriologic study of rectal swab cultures was made from children suffering from diarrhea and from control subjects without enteric symptoms. Immediately after the specimen was collected the swab was placed in preservative broth and brought to the

laboratory within one or two hours. Plates of MacConkey agar and SS agar were inoculated and the swab then was placed in tetrathionate broth for enrichment. The plates were examined the following morning and the enrichment medium was streaked on an SS plate. Tryptose phosphate broth was inoculated from selected colonies. When growth became evident this culture was used to inoculate the carbohydrate series and other test media. Most of the *Shigella* species and *Salmonella* types were considered as known pothogens, while *Paracolobactrum*, *Proteus*, *Alcaligenes*, *Pseudomonas* were considered possible pathogens. The coliform bacilli usually present in the intestinal flora were considered as non-pathogens. It was not possible to check the coliforms to ascertain whether any were of the types reported to be pathogenic.

A total of 330 cultures were studied from 111 infants with diarrhea (92 under one year and 19 between one and two years) and 224 cultures from 186 control infants (135 under one year and 51 between one and two years). In Table II, the types of bacteria isolated and their relative distribution in per cent of the total isolations for the various age groups are presented. An "isolation" was counted as positive but once, whether the organisms were cultured one or more times from the same patient. Also listed are the number of patients from whom isolations were made.

Results

Under 2 months of age, *Salmonella* was isolated from one patient (comprising three per cent of the total isolations) and *Shigella* from none. *Paracolobactrum* isloations were more common in the control patients than in the patients with diarrhea especially *Paracolobactrum aerogenoides*. *Proteus morganii* isolations definitely were more frequent in the diarrhea subjects than in the controls. *Alcaligenes* was isolated in the controls but not in the infants with diarrhea. *Pseudomonas* isolation was slightly greater in infants with diarrhea. The proportion from which coliforms only were isolated was practically the same in both groups.

In the 2 to 6 month age group *Salmonella montevideo* was found in one patient without diarrhea but this patient subse-

TABLE 2

Types of bacteria isolated with relative distribution (per cent of total isolations) and number of patients in various age groups of 111 infants with diarrhea (330 cultures) and 186 control subjects (224 cultures).

Age Groups	Salmonella	Shigella sonnei	Shigella paradysenteriae	Paracolobactrum aerogenoides	Paracolobactrum coliforme	Paracolobactrum intermedium	Proteus mirabilis	Proteus morganii	Proteus rettgerii	Proteus vulgaris	Alcaligenes	Pseudomonas	Coliforms only
0–2 mo.													
Control (29 pts.)													
Isolations	0	0	0	24	19	3	14	17	4	14	7	10	38
No. of subjects	0	0	0	7	5	1	4	5	1	4	2	3	10
Diarrhea (39 pts.)													
Isolations	3	0	0	3	10	8	18	44	8	15	0	18	36
No. of subjects	1	0	0	1	4	3	7	17	3	6	0	7	14
2–6 mo.													
Control (50 pts.)													
Isolations	2	0	0	16	14	14	22	30	6	16	2	12	22
No. of subjects	2	0	0	8	7	7	11	15	3	8	1	6	11
Diarrhea (37 pts.)													
Isolations	11	3	0	24	19	3	16	22	3	16	3	30	14
No. of subjects	1	1	0	9	7	1	6	8	1	6	1	11	5
6–12 mo.													
Control (56 pts.)													
Isolations	0	0	0	9	16	2	14	36	4	11	4	13	23
No. of subjects	0	0	0	5	9	1	8	10	2	6	2	7	13
Diarrhea (16 pts.)													
Isolations	13	0	6	13	44	6	25	50	0	13	6	19	6
No. of subjects	2	0	1	2	7	1	4	8	0	2	1	3	1
12–24 mo.													
Control (51 pts.)													
Isolations	4	0	2	8	8	2	10	16	4	2	2	14	43
No. of subjects	2	0	1	2	4	1	5	8	2	1	1	7	22
Diarrhea (19 pts.)													
Isolations	5	16	0	11	16	32	11	47	11	11	16	21	5
No. of subjects	1	3	0	4	3	6	2	9	2	2	3	4	1

quently was found to have *Salmonella* meningitis (Beene *et al.*, 1951). *Shigella sonnei* was isolated from one patient with diarrhea. No striking difference was found in the proportion of isolations of *Paracolobactrum* (except *intermedium*) and *Proteus* species. *Pseudomonas* was isolated more commonly in the patients with diarrhea than in the control subjects and the opposite was true for "coliforms only."

In the 6 to 12 month age group, *Salmonella* and *Shigella paradysenteriae* were found in the diarrhea but not in the control group. *Paracolobactrum*, *Proteus mirabilis* and *morganii* isolations were more frequent in patients with diarrhea. In the patients with diarrhea a marked decrease was observed in the coliforms as the only organism isolated.

In the 12 to 24 month age group, isolations of *Salmonella* species were not much different in the diarrhea and control patients. *Shigella sonnei* was isolated from three of nineteen diarrhea patients. *Paracolobactrum* and *Proteus* species tended to be isolated more frequently in the diarrhea patients than in the control subjects. Coliforms again were the predominant organisms isolated in the control patients who did not have enteric symptoms.

The results may be compared with the findings obtained in two other series wherein the same methods of isolation were employed. One study included 372 cultures on 163 different patients (107 with diarrhea) carried on in Galveston in 1945–1946 and the other in San Antonio where 1,150 cultures were made on 376 patients with diarrhea. In Table III the results are given from study of 1,814 swab specimens from patients with diarrhea and 497 from control subjects. The data in this table also include findings from patients over two years of age although the greater portion of the patients with diarrhea were children less than two years of age.

In the two series of studies in Galveston the proportion of isolations of *Salmonella* and *Shigella* was similar, on the other hand in San Antonio these types were isolated more than twice as commonly. *Paracolobactrum* species were found somewhat more frequently in the patients with diarrhea than in the control subjects. In the second Galveston series *Proteus* species were found much more commonly. It is known that these organisms are well known invaders of damaged tissue, hence even

TABLE 3

Relative frequency in per cent of organisms isolated from 2311 stool cultures (1814 on patients with diarrhea and 515 on controls) obtained from 1074 subjects (615 patients with diarrhea and 459 controls).

		Salmonella	Shigella	Paracolo-bactrum	Proteus	Alcali-genes	Pseu-domonas	Coliforms only
First Galveston Series 1945–46	Control (56 pts.) 56 cultures	0	2	41	51	4	14	21
	Diarrhea (107 pts.) 316 cultures	9	11	48	58	5	7	14
Second Galveston Series 1947–50	Control (403 pts.) 459 cultures	1	1	32	36	6	11	35
	Diarrhea (129 pts.) 348 cultures	7	9	46	78	8	19	14
San Antonio Series 1946–48	Control	—	—	—	—	—	—	—
	Diarrhea (379 pts.) 1,150 cultures	18	23	—	—	—	—	26

when not primarily causative their presence may incite a more serious infection. *Proteus* colonies were numerous in a heavy growth when present on the plates from patients with diarrhea but only a few colonies would appear on plates from control subjects. Often these organisms were found to increase greatly when the incidence of *Shigella* or *Salmonella* decreased.

Inasmuch as *Alcaligenes* and *Pseudomonas* were not significantly different in the two groups it is not possible to throw heavy suspicion on these bacteria as pathogens in infantile diarrhea. Frequently the growth of *Pseudomonas* increased with the duration of the diarrhea.

Discussion

There is scarcely room for doubt that many bacterial organisms are implicated in diarrheal episodes in infants and children. On the basis of our studies the true significance of bacterial agents may seem somewhat in doubt in that the results of rectal swab cultures in patients with diarrhea and control subjects show so little difference. It is known that the bacterial

flora of the upper respiratory passages may be relatively the same in patients with an upper respiratory tract infection and in control subjects. It does not seem that one may infer that the causative agent is a virus simply because bacteria cannot be implicated unequivocally in one or another single case or in an epidemic of diarrhea. Advancements in the field await the development of better techniques both for the isolation of bacterial as well as viral causative agents.

Summary

Periodic rectal swab cultures from 111 infants with diarrhea and 186 control subjects under two years of age revealed that *Salmonella* types and *Shigella* species were uncommonly found in young infants, but in infants with diarrhea isolation of these organisms increased with increasing age. The frequency of isolation of *Paracolobactrum*, *Proteus* species, *Alcaligenes* and *Pseudomonas* in the two groups of infants was so variable from the viewpoint of both age of patients and the condition of diarrhea that it is not possible to draw any definite conclusion as to their pathogenicity in infantile diarrhea.

B. Sensitivities to Selected Antibiotics of Enteric Bacilli

In the hope that one or more of the newer and rapidly developing antibiotic agents would be valuable therapeutically, the effort was made to study *in vitro* sensitivities of enteric bacilli isolated from diarrhea patients. In addition to the organisms isolated from our patients nine of the Salmonella types studied were obtained from Doctor P. R. Edwards of the Communicable Disease Center, Chamblee, Georgia. For sensitivity determinations, whenever practicable, the plate streak method was employed. To prepare the plates, appropriate dilutions of each antibiotic were added to bottles of tryptose agar just before pouring. The plates were marked off into 8 sectors and inoculations were made using a cotton-tipped applicator dipped into a 6–8 hours broth culture of the organism to be tested. The tests were incubated for 18 hours at room temperature. If the organisms were spreaders (e.g. *Proteus*) it was necessary to employ a liquid medium. In such instances tryptose broth was used. The antibiotic was serially diluted in 0.5 ml. of broth, after which

1.5 ml. of 1:1000 dilution of a young broth culture was added. The tests were incubated 18 hours at room temperature. The 50 per cent end points were determined by a modification of the method described by Heden.

Results

The results of the comparative sensitivity tests which are expressed as the mean of the 50 per cent end point determinations are presented in Table IV.

TABLE 4

Sensitivities to selected antibiotics: Means of 50 per cent end-point determinations expressed in micrograms per milliliter for 105 strains of enteric organisms.

No. Strains Tested	Organism	Neomycin	Aureomycin	Chloromycetin	Streptomycin
10	Proteus mirabilis	12.18	13.73	19.38	15.51
7	Proteus morganii	10.01	3.27	6.28	3.37
11	Proteus rettgeri	16.64	11.98	10.38	6.17
10	Proteus vulgaris	8.03	4.22	15.21	5.79
5	Pseudomonas	22.62	2.30	18.59	8.23
32	Salmonella	12.69	1.06	2.75	1.95
14	Sh. paradysenteriae	23.29	0.33	0.57	5.07
5	Shigella sonnei	17.58	1.32	1.98	6.46
6	Escherichia	6.35	0.33	2.20	1.09
5	Aerobacter	5.83	0.36	4.42	0.65

As indicated in Table IV the amount of neomycin required to inhibit *Shigella* and *Pseudomonas* was very high. Coliforms proved more sensitive to this antibiotic than did the non-lactose-fermenting enterics. Aureomycin was required in much greater amount to inhibit members of the *Proteus* group than for the other organisms studied. Its effectiveness in inhibiting growth of *Shigella paradysenteriae*, *Escherichia* and *Aerobacter* was in the same order of magnitude and was greater than for the other organisms. Chloromycetin was ineffective in decreasing the growth of the *Proteus* group and *Pseudomonas*. *Shigella paradysenteriae* was the most sensitive of the organisms studied. Streptomycin was required in largest amount to inhibit *Proteus mirabilis*. The greatest degree of sensitivity was shown toward *Aerobacter*, *Escherichia* and *Salmonella* in the order named.

In regard to the relative sensitivities of the individual organisms in relation to the various antibiotics the following points were noted. *Proteus mirabilis* was slightly more sensitive to neomycin than to the other antibiotics tested. *Proteus morganii* was somewhat more sensitive to neomycin than was *Proteus mirabilis* but much more sensitive to the other 3 agents. *Proteus rettgeri* was quite resistant but was most sensitive to streptomycin. *Proteus vulgaris* was more senstitive to neomycin than *Proteus mirabilis* and was fairly sensitive to aureomycin. *Pseudomonas* was quite resistant to neomycin and chloromycetin. The *Salmonella* types were sensitive to smaller concentrations of both aureomycin and chloromycetin than to neomycin. The organisms tested also were sensitive to streptomycin with the exception of 2 strains which had a 50 per cent end point of more than 35 micrograms per milliliter. *Shigella paradysenteriae* was extremely resistant to neomycin but indicated a high degree of sensitivity to aureomycin and chloromycetin. *Shigella sonnei* was only slightly less resistant to neomycin than was *Shigella paradysenteriae* and was more resistant to the other antibiotic agents. Of all the organisms tested, the coliforms, *Escherichia* and *Aerobacter* were the most sensitive to neomycin.

Discussion

A wide variety of organisms possibly may be related etiologically in diarrhea, yet when the results of rectal swab cultures from infants with diarrhea are compared with those from control subjects it is somewhat difficult to implicate more than a few bacterial species as being pathogenic. By the same token it is equally disconcerting that certain antibiotic agents do not have a wide spectrum of action against known and possible enteric pathogens. Aureomycin seemed to show greater antibiotic activity than any of the other agents. Chloromycetin also seemed to be effective against several strains and streptomycin showed especial activity for *Salmonella* and also *Proteus morganii*. Neomycin was particularly ineffectual. As this work was being carried out, simultaneous *in vivo* studies were being made (herein reported) to ascertain whether the results corresponded with the *in vitro* findings.

Summary

Resistance studies to neomycin, aureomycin, chloromycetin and streptomycin *in vitro* on 105 strains of enteric bacilli isolated from 85 patients with diarrhea disclosed: 1. *Proteus mirabilis* and *Proteus vulgaris* were resistant to neomycin; 2. *Pseudomonas* exhibited a sensitivity to aureomycin; 3. *Salmonella* types were most sensitive to aureomycin and chloromycetin; 4. *Shigella* strains were quite sensitive to aureomycin and chloromycetin but very resistant to neomycin; 5. *Escherichia* and *Aerobacter* were very sensitive to aureomycin and of all the organisms studied were the most sensitive to neomycin.

C. Effect of Administration of Neomycin on Enteric Flora in Patients with Diarrhea:

Periodic rectal swab cultures were made on 25 of 31 children with diarrhea who were receiving a therapeutic trial with neomycin. The usual oral dosage was 0.1 gm. per kg. body weight per day. *Shigella* species had been isolated from 8, *Salmonella* from 3 and no accepted pathogens from 14 of the 25 patients. Representative data are presented in Table V for four of the cases studied.

Results

In case R. D., within a relatively short time after the neomycin had been administered, the rectal swab culture showed no growth. Three days after neomycin had been discontinued a heavy growth of enteric bacilli was again present. The findings in case S.M.J. were typical of the results obtained in all 8 children with *Shigella* diarrhea. After the administration of neomycin, *Shigella* was found in pure culture. Although there was a gradual reduction in the number of organisms present, it was necessary to give sulfadiazine before this organism was eliminated from the stool. The findings in relation to case M.L.F. demonstrated that the *Proteus* species gradually were eliminated from the cultures. Previous *in vitro* studies indicated these organisms to be relatively resistant to neomycin. It is well known that there is a tendency for secondary invasion of the *Proteus* species in chronic diarrhea and it appears this secondary invasion is diminished by neomycin. In reference to case S.A.B.

TABLE 5

Representative data regarding bacterial isolations in children with diarrrhea given neomycin.

	CASE RD	CASE SMJ	CASE MLF	CASE SAB
Initial bacterial isolations	Paracolobactrum coliformi Proteus rettgeri Aerobacter Eschericheria	Sh. paradysenteriae II. Proteus morganii Proteus rettgeri Escherichia	Proteus rettgeri Proteus vulgaris Escherichia	Paracolobactrum aerogenoides Paracolobactrum intermedium Proteus vulgaris Aerobacter
Days after neomycin begun		Sh. paradysenteriae II.	Proteus rettgeri Proteus vulgaris	Pseudomonas (scant)
1	Proteus coliforme) scant Pseudomonas)		Escherichia Aerobacter	
2	No growth	Sh. paradysenteriae II.	Proteus vulgaris Escherichia	Pseudomonas (abundant)
3	No growth		No growth	
4	*			
5		Sh. paradysenteriae		
6		Sh. paradysenteriae	*	
7	Proteus aerogenoides Proteus vulgaris Aerobacter	†	Pseudomonas Escherichia Aerobacter	Pseudomonas Aerobacter Escherichia
8		No growth		
9		No growth		
10	Proteus morganii Proteus vulgaris Aerobacter			

*Neomycin discontinued.
†Sulfadiazine given.

it is apparent that *Pseudomonas* is resistant to neomycin when administered to patients with diarrhea.

Summary

Bacteriologic studies from rectal swab cultures were made on 25 children with diarrhea who were given neomycin orally. In general, there was a marked reduction in the number of organisms isolated. *Proteus* species tended to be eliminated when neomycin was administered although *in vitro* sensitivity tests showed these organisms to be resistant to neomycin. There was no effect on *Pseudomonas*. The most striking observation was the persistence of the pure cultures of *Shigella* in the 8 children with diarrhea from whom this organism had been isolated initially.

III. Therapeutic Approach to Problem of Infantile Diarrhea

Any mother, nurse or physician may make the diagnosis of "diarrhea"—whether there be a slight change in the frequency and consistency of the stools or a large, watery, green, slimy, bloody bowel movement, or the development of a severe shock-like state in association with numerous watery stools. The outstanding feature seems to be not so much to diagnose "diarrhea" but to recognize and anticipate the implications involved when there is a change in the character of an infant's stool.

During one period of approximately six months 65 infants with "diarrhea" were seen in the nurseries, wards and outpatient clinic at The University of Texas Medical Branch Hospitals. There were no instances of serious dehydration or acidosis and no deaths occurred. Under these conditions it would be difficult to draw definite conclusions regarding almost any therapeutic regimen for infants with diarrhea, for, undoubtedly, similar results would have been obtained had any one of a host of anti-diarrheal agents been employed. It is an experience such as this that forms the basis of claims for most of the "theapeutic adjuncts" used in diarrhea. In reviewing the case histories of many infants with diarrhea which lead to severe dehydration and acidosis, numerous drugs and remedies had been employed.

Shortly after the diarrhea study had been instituted by the Child Health Program, Darrow reported his significant metabolic and clinical studies in infantile diarrhea. Of particular importance was the role of the potassium ion in relation to the maintenance of water and electrolyte balance. A portion of the studies carried on by Darrow and his colleagues were under the auspices of the Child Health Program. It was found that under conditions of high temperature and/or humidity, the fluid requirement of infants with diarrhea definitely is increased by as much as 50–100 ml. per kg. per day (Darrow, *et al.*, 1949). The extent of the potassium deficit in diarrhea was realized from the study of Gamble, Wiese and Hansen, who found that it was necessary to administer relatively large amounts of potassium to effect recovery from cardiac failure associated with extreme hypokaliemia. In our theapeutic studies the basic pattern of approach has been that of Darrow, wherein potassium containing replenishment fluids have been employed. Attempts also have been made to evaluate various chemotherapeutic and antibiotic agents, the results of which are reported herewith.

A. Comparative Studies

1. Sulfonamides

The development of non-absorbable sulfonamides suggested their use as possible therapeutic agents in diarrhea. Attempts were made to determine the efficiency of sulfasuxidine, sulfaguanidine, and sulfathalidine as well as sulfathiazole and sulfadiazine in "run of the mill" diarrheal cases. The dosages used were those usually employed for these drugs. The results were compared with those obtained using the same therapeutic measures without sulfonamide administration, *i.e.*, maintenance of fluid and electrolyte balance. At the beginning, alternate cases were not used but conditions were kept comparable in the sulfonamide treated patients and in those who did not receive sulfonamides. In general, the duration of the diarrhea and the severity were similar in both groups. All the infants studied were under two years of age, although the greater portion were less than six months of age. From 1944 to 1946 there were 122 infants studied. The results are summarized in Table VI.

TABLE 6

Results of treatment with sulfonamides in infants with diarrhea and in control subjects.

*Sulfonamide**					*Control*				
No. of Patients	Good	Fair	Poor	Death	No. of Patients	Good	Fair	Poor	Death
				1944					
5	5	—	—	—	1	1	—	—	—
				1945					
21	15	2	3	1	6	6	—	—	—
					16	14	2	—	—
				1946					
8	6	1	1	—	14	11	1	2	—
12	10	1	1	—	3	3	—	—	—
†18	11	5	2	0	18	10	4	4	0
				TOTAL					
64	47	9	7	1	58	45	7	6	0

*Sulfasuxidine, sulfaguanidine, sulfathiazole, sulfadiazine.
†Sulfathalidine series.

In the routine types of diarrhea as seen in the out-patient dispensary or hospital it is apparent from the data in Table VI that the administration of none of the sulfonamides had much effect on the course of the illness. A further attempt was made to evaluate sulfathalidine[1] more critically by using the alternate case method for 36 infants. In the 18 subjects with diarrhea and the 18 control patients there were no deaths. The proportion of good, fair and poor results were about the same in both groups. Hence, the overall results for 64 infants with diarrhea treated with a sulfonamide were not noticeably different from those for the 58 infants with diarrhea who were not given a sulfonamide.

2. p-amino-phenyl-(1-phenyl-2-benzoyl-ethyl)-sulfone. (Nu 404)[2]

Evaluation of this drug was part of a clinical study carried on in San Antonio. In this phase of the project there were 150 infants with diarrhea. Of those given the drug forty-four were treated on an out-patient basis and twenty-two as hospitalized patients. The dosages were 0.125 to 0.25 gm. per kg. per day in divided doses every four hours. At the same time, 84 patients (35 out-patient and 49 hospitalized patients) received the same therapy with the exception of the experimental drug.

[1]Kindly furnished by Sharp and Dohme, Philadelphia, Pa.
[2]Kindly furnished by Hoffman-LaRoche, Inc., Nutley, N. J.

The results may be summarized as follows: The administration of the drug did not affect the course of the disease. No distinct difference in the duration of the diarrhea was found in the two groups. The clinical impression was gained, however that infants who were seriously ill and showed positive cultures for *Shigella* appeared to respond more rapidly after the drug was administered especially at the higher dosage level. On this basis it was felt that the drug had sufficient merit to extend the study. No influence on *Paracolobactrum* or *Salmonella* isolations was noted and there was no change in the clinical condition of these patients which could be attributed to the drug.

3. Neomycin

With the early observation by Poth and co-workers (1950) that neomycin was effective in sterilizing the gut for surgical procedures, it seemed logical to study the possible therapeutic effectiveness of this agent in infants suffering attacks of acute diarrhea. There were 56 infants in the group studied, 31 of whom were given 0.1 gm. per kg. neomycin per day in 6 equal doses for a period of from 3 to 8 days. In some instances rectal swab cultures became negative or growth was scanty. There was no difference, however, in regard to the severity or length of an attack of diarrhea. There were no deaths.

Discussion

The tendency is to compare therapeutic results obtained in one institution or locality with those reported from others. Sometimes results in a given year are compared with those of another year in the same institution. In lieu of nothing better this may be allowed, but from the viewpoint of validity of comparative data, so many variables (ability of physicians and nurses, socio-economic status of patients, institutional facilities, season of year, type and virulence of organisms involved, nutritional condition of patient) are possible and cannot be controlled so that it is difficult indeed to reach unequivocal conclusions. In other words, few if any clinical studies have been made wherein a single therapeutic agent has been the only variable in alternate cases while all other features have been kept constant. Even if a very well controlled study is carried on, the

results obtained are not necessarily applicable to the infantile diarrhea problem in another locality. Insofar as is known, no chemical or antibiotic agent has been found to give spectacular results in a large number of infants with diarrhea. In view of the high mortality in many localities sustained efforts should be directed toward finding agents with a wide spectrum of effectiveness.

B. Clinic and Office Management:

In considering the infantile diarrhea problem first attention, prophylactically or therapeutically, must be directed to the metabolism of water and electrolytes. The greatest danger to the patient lies in the development of dehydration and alterations in the acid-base balance. Not every child who has "running of the bowels" develops severe dehydration, nevertheless every child who has loose stools is eligible to develop a deranged physiologic state. It is the purpose of this part of the paper to report the results of out-patient management of infantile diarrhea. The study was made possible by the wholehearted cooperation of the Sisters of the Incarnate Word of the Santa Rosa Hospital of San Antonio. The main objective of the therapeutic regime was to prevent the development of dehydration and electrolyte depletion. Because of the limited bed capacity it was necessary to handle many infants on an out-patient regimen who were quite ill and ordinarily would have been hospitalized. There were 120 infants observed within a period of 3½ months during the height of the diarrheal season (May 15 to Sept. 1, 1947). The principles of the therapeutic regimen are those of Darrow and co-workers.

1. General measures—

a) Thorough examination including weight and temperature; then daily visits until condition is satisfactory.

b) Careful explanation of regime to mother supplemented with written directions (English and Spanish).

c) Urinalysis and blood count.

d) Rectal swab culture when first seen and 24 hours later. The specimen was placed directly in broth and brought to the laboratory within an hour.

e) Accurate record (by both mother and physician). (1 Number and character of stools. (2) Actual fluid intake, type and content.

2. Details of regime—

a) First 24 to 48 hours—total fluid 200–300 ml. per kg. Composition of fluid: boiled tap water, sodium chloride— 2.0 gm. per quart, carbohydrate (Karo, dextrose, sugar or Dextrimaltose)—2 ounces per quart. (To ⅓ of the patients 1–2 gm. potassium chloride was added to each quart of fluid). Administration: calculated daily requirement divided into 8 equal parts and given every 3 hours.

b) Subsequent days—total fluids 150 to 200 ml. per kg. per day (when diarrhea subsiding, patient afebrile and well hydrated). Composition of fluid: skimmed milk to supply 20 cal. per kg. (skimmed milk contains 0.4 cal. per ml. or 12 cal. per oz.) and boiled tap water containing 5 per cent carbohydrate (potassium chloride 1.0 to 2.0 gm. if indicated). Administration: calculated amount divided into 8 equal parts and given every three hours.

E.g. 5 kilograms (11 pounds) infant.

Total fluid	5×200	= 1000 ml.
Skimmed milk 5×20 cal. $= 100 \div 0.4$		= 250 ml.
Water (with 5 per cent CHO)		= 750 ml.

8 feedings $8\overline{)1000 \text{ ml.}}$ 125 ml.

or 4 ounces every 3 hours, 8 times in 24 hours.

c) *Gradual resumption of feeding*—increase skimmed milk usually 10–30 cal. per kg. per day depending upon condition of infant; combine with water containing 5 per cent carbohydrate to meet daily fluid requirement. When once receiving the full skimmed milk mixture, this was maintained for several days before an evaporated milk or whole milk mixture was given.

d) *Supportive measures*—supplements of Vitamin B and C were usually given and occasionally, Vitamin K was injected intramuscularly to the more seriously ill infants.

Results

Of the 120 infants treated in the out-patient clinic it was necessary to admit 9 to the hospital. Only one infant died—a month old premature weighing 4 pounds, 14 ounces (2190 gm.). Of the 9 infants admitted to the hospital 3 were admitted within 24 hours after the initial examination. There were 15 infants who were not brought back to the dispensary; however, careful check of death certificates indicated none of these succumbed during the period of study. It was learned that failure to return generally was due to improvement of the patient so that the parents did not consider further medical attention

necessary. Detailed consideration of the results is included in the discussion.

C. Hospital Management:

There were 71 infants in this group, 59 of whom were admitted directly to the hospital and the remaining 12 patients (1 to 6 weeks of age including 10 prematures) developed diarrhea while in the hospital.

Age distribution:

Six months or under	56	(16 prematures)
Six months to 1 year	13	
One to two years	2	

Clinical evaluation of severity:

Very severe or critical	20
Severe	29
Moderate	13
Mild	9

Carbon dioxide combining power of the serum: The determination was made on 51 patients at the time of admission with the following distribution:

HCO_3 mm. per liter	No. of Patients	Clinical Condition			
		Very severe	Severe	Moderate	Mild
0–5	6	5	1	..	..
6–10	13	6	6	1	..
11–15	21	6	13	2	..
16–20	5	..	3	1	1
21–25	5	..	1	4	..
26–30	1	..	1	..	..

Associated infections and conditions:

Acute otitis media—6, bilateral suppurative mastoiditis—1, septicemia—1, congenital syphilis—2, pneumonia—6, thrush—7, acute bronchitis—1, abscesses in thigh—1, severe malnutrition—9, congenital heart disease—3, arthrogryposis congenita multiplex—1, mongolism—1, tuberculosis—1, pertussis—1 and scabies—frequent.

Details of routine hospital management:

1. *First 24 hours*

a) Total fluids: 200–300 ml. per kg. 20–30 ml. per kg.—0.85% NaCl, i.v.—rapidly (10–20 min.) 20–30 ml. per kg.—plasma or whole blood i.v.—rapidly (15–30 min.) 90–140 ml. per kg.—5 per cent dextrose in water—

slow i.v. drip (15–20 drops per min., slower later) 70–100 ml. per kg.—Darrow's[3]—K-Bicarbonate or K-Lactate solution—given by clysis at rate of 8–12 drops per min. *after* infant urinates.

b) Nothing by mouth.

c) When the CO_2 combining power was below 15 volumes per cent, (7.0 mM per liter) a four per cent solution of sodium bicarbonate was given intravenously. The empirical formula used in calculating the amount of $NaHCO_3$ to be given was — gms. $NaHCO_3 = 1/38 \times$ weight (kg.) $\times$ volumes per cent to be raised (usually 10 volumes per cent).

d) Careful record of intake including the amount and type of solution.

e) Frequent evaluation of patient by physician and careful observation by nurse. After the first 24 hours, if the infant had poor skin turgor or other evidences of dehydration, fever, marked distention, or frequent watery stools, the same routine was followed for the second 24 hours. In several instances parenteral fluids were used exclusively for 3 to 4 days; usually, however, oral feedings could be started at the end of the first 24 hours.

2. *Second 24 hours*

a) Total fluids: 150–200 ml. per kg. Darrow's solution 1 part. Dextrose (5 per cent) in distilled water 2 or 3 parts. The fluid requirement was calculated and the total amount was divided into 8 equal portions and given every three hours.

b) Careful record of intake was made at 8 to 12 hour intervals and supplementary fluids, if needed, were given by clysis or slow intravenous drip.

c) As indicated blood transfusions were given.

3. *Third 24 Hours*

If the clinical condition warranted then the skimmed milk mixture with added carbohydrate was given to supply 150–200 ml. per kg. total fluid as outlined under out-patient management. Of course, when the calculated required fluids were not consumed then parenteral fluids were administered.

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[3]Potassium containing replenishment fluids were kindly furnished by Upjohn Company on an experimental basis. The compositions were:

		(gms. per 100 ml. water)
K-Bicarbonate Solution—	$NaHCO_3$	0.44
	KCl	0.27
	NaCl	0.4
K-Lactate Solution	—Na lactate (racemic)	0.59
		(53 ml. molar solution)
	KCl	0.27
	NaCl	0.4

4, *Supportive Measures.*

a) Parenteral vitamins B complex and C were given for the first 2 or 3 days; then a water-miscible multiple vitamin preparation was administered orally.

b) Vitamin K was injected intramuscularly daily to the severely ill infants and to those with prolonged diarrhea.

c) Calcium gluconate, 1–2 gm. daily was added to fluid mixture of all infants with severe or prolonged diarrhea until a skimmed milk formula was started. If manifest tetany developed 0.5 to 1.0 gm. calcium gluconate was given as a 5 per cent solution very slowly intravenously (not intramuscularly).

d) Antibiotic and chemotherapeutic agents were given as indicated. Premature and small infants or those with prolonged and severe diarrhea were given penicillin 100,000 units prophylactically (not therapeutically for the diarrhea *per se*).

5. *Miscellaneous features*

Whenever possible, blood was obtained from the infants for determination of the carbon dioxide combining power, chloride, sodium, and potassium.

Results:

The duration of the diarrheal period and the length of the hospital stay were ascertained but it was felt that this information had no particular value because data regarding similarly afflicted children treated by other routines were not comparable. Of the 71 hospitalized patients there were 6 deaths. The summaries of these fatal cases are presented:

Case 1.—R. B., white premature, 25 days old, weight 1,950 gms. 4 lbs., 4½ oz.), seemed to be doing well but suddenly expired. Autopsy revealed multiple pulmonary pyogenic abscesses. Diagnosis—aspiration pneumonia.

Case 2.—S. S., Mexican female 6 months old, weight 4,760 gms. (10 lbs., 8 oz.). On admission the child had convulsions and a temperature of 106° F. There was evidence of bronchopneumonia. The serum CO_2 combining power was 11.8 millimols per liter. The infant developed tetany with a serum calcium level of 6.5 mg. per 100 ml. Death occurred 22 hours after admission. Autopsy revealed acute enteritis with bronchopneumonia.

Case 3.—S. G., Mexican female, 4 months old, weight 4,000 gms. (8 lbs., 12 oz.) expired within 30 minutes after arriving at the hospital. Temperature was 106° F., dehydration severe, in coma. The serum carbon dioxide combining power was 12.3 millimols per liter. Autopsy revealed acute enteritis, pulmonary edema and bronchopneumonia.

Case 4.—P. F., Mexican male, 6 months old, weight 7,200 gms. (16 lbs.). On admission was markedly dehydrated and comatose, expired within 30

minutes of admission. Autopsy was not permitted. An attempt was made to aspirate blood from the heart but purulent fluid was obtained. Subsequent aspiration revealed empyema. The total protein value of the aspirated fluid was 5.13 gm. per cent. Many pus cells were seen on smear. Culture revealed *Staphylococcus albus*.

Case 5.—G. H., Mexican female, 30-day-old infant, weight 2,200 gms. (4 lbs., 13 oz.), died 72 hours after admission. The infant was believed to be progressing satisfactorily and prior to death had been placed on a skimmed milk mixture supplying 20 cal. per kg. per day. On day of death there were 2 soft yellow stools. Autopsy revealed extensive bilateral bronchopneumonia.

Case 6.—D. H., Mexican male, 4 months old, with florid congenital syphilis. There was marked bone destruction, and persistent albuminuria in spite of penicillin therapy. An exfoliative dermatitis developed while on penicillin therapy. The infant was kept in the hospital for about two months during which time he had two episodes of diarrhea. He responded well to the routine treatment on the first occasion but died suddenly during the second episode of diarrhea. Electrolyte studies obtained on the day of death were: Na 119, Cl 88, K 7.8 meq. per liter. Autopsy revealed bronchopneumonia, acute enteritis, acute hepatitis, acute nephritis with right hydronephrosis and acute toxic changes in all viscera.

The following complications were noted among the hospitalized patients:

Recurrence of diarrhea—7, pneumonia—6 (aspiration—3), thrush—12, generalized edema—6, convulsions—4, icterus—3, urticaria—2, abscess—5 (thigh—4, back—1), thrombophlebitis —1, intussusception—1, anemia—frequent.

Stool cultures:

The results of stool cultures disclosed no relationship between the nature of the organisms isolated and the mortality data or the character of the complications.

Drug Administration:

Complicating and associated infections were treated with penicillin. Approximately one-third of the hospitalized patients were given an experimental insoluble sulfonamide (Nu 404). It so happened that one of the patients who received this drug died. It was the feeling of the observers that the chief value of the medication was with patients from whom *Shigella* had been isolated.

Serum electrolyte studies:

Serum electrolyte determinations for sodium, potassium and chloride were obtained one or more times on 20 patients. The serum potassium was less than 3.5 milli-equivalents per liter in 6 infants, and the lowest value found was 2.0 milli-equivalents per liter. In most instances the blood was obtained at the time of admission to the hospital. It has subsequently been learned that on rehydration of the patient the serum potassium values frequently fall to very low levels in the severely dehydrated patient with prolonged diarrhea. Balance studies were not carried out on any of these babies, hence the extent of the mineral depletion was not known.

Discussion of Therapeutic Studies:

It is rather remarkable that when one attempts to study the therapeutic value of particular drugs and methods of treatment that such a great variation in the results occurs. Although all the patients studied seemed to have been classified properly as—diarrhea, enteritis or dysentery—a great difference in the outcome was noted. In the series where the attempt had been made to evaluate sulfonamides and neomycin, a total of 95 infants had been given the respective drugs and 83 infants with diarrhea had been treated quite comparably yet only one death occurred in the total group. On the other hand in the 71 infants hospitalized because of the severity of the child's condition, six deaths occurred. It may be of some interest to discuss the mortality data concerning the diarrhea study which was carried on in an area where deaths from infantile diarrhea are a problem of major importance to the public.

Consideration may be given to mortality data for the combined dispensary and hospitalized patients in relation to the diarrhea problem in the city.

Total experience with infants		182
Infants in out-patient clinic		120
Infants subsequently admitted to hospital (3 within 24 hours, 6 over 24 hours)	9	
Infants failed to return for follow-up	15	
Total infants followed strictly as out-patients	96	
Infants hospitalized		71
Infants developing diarrhea in hospital	12	
Referred to hospital from out-patient	9	
Referred directly to hospital	50	
Total deaths	6	

One could consider the mortality to be 8.5 per cent on the basis of the 71 hospitalized infants (6 deaths). If one chose to deduct the two infants who were so critically ill that they succumbed within one-half hour after being admitted even before therapy was instituted, then the mortality could be calculated as 5.9 per cent. Likewise, if one chose to consider only the 59 infants who were referred from the outside (excluding the 12 hospitalized infants) then the rate could be considered to be 6.5 per cent excluding the almost immediate deaths. One also may consider the total number of patients studied as 182 with 4 deaths (exclusive of the immediate deaths); then the rate would be 2.2 per cent.

It was believed that something further could be learned regarding the efficiency of the therapeutic measures in relation to mortality from infantile diarrhea by studying death certificates and records of the City Health Department as well as the records from the Santa Rosa Hospital. Admittedly, high infantile diarrhea death rates occur in this area particularly during the summer months. According to the San Antonio City Health Department records for the years of 1945 and 1946 the number of cases reported were 621 and 565 respectively, the deaths were 209 and 122 respectively. There were 380 cases of diarrhea reported for the first 8 months of 1947 with a total of 60 deaths, only 6 of which occurred in the 182 cases cared for by the study group. It is of some significance that in the two months following cessation of the co-operative clinical study there were 11 deaths in 35 infants hospitalized because of diarrhea. From the viewpoint of the Child Health Program this pilot study seemed to point out that concerted educational efforts at the physician, nurse and hospital level can go far to solve the diarrhea problem.

IV. General Discussion

In this day of miracles in medicine our admiration is apt to be centered on the spectacular discoveries of the modern age. Sometimes we forget that dividends in life saving and life prolongation resulting from recent advancements are small compared with those which have occurred from the proper feeding of infants by reducing infantile diarrhea deaths. In sections of

the country where high infant death rates exist one is apt to find that many deaths result from intestinal disorders. Knowledge concerning prevention of intestinal disorders in infants is not nearly as widespread as it should be. More significant than this from the viewpoint of the physician is that in the event diarrhea occurs it is possible to reduce death rates by emergency treatment with replenishment fluids and electrolytes. Our studies revealed that maintenance of fluid and electrolyte balances in the early stages of diarrhea may go far in preventing the development of severe dehydration and acidosis and the resulting death. Equally significant on the basis of the studies herein reported as well as those in the current literature, is the ineffectiveness of any known single agent or combination of chemical or antibiotic substances in curing diarrhea in infants. It must be emphasized that every effort should continue to be directed to finding truly effective antidiarrheal agents. In the meantime, therapeutic efforts should be based on the application of physiologic principles directed toward correcting the deranged metabolic states. At present this is our great advancement in the therapy of diarrheal infants.

V. Summary and Conclusions

The Child Health Program of The University of Texas carried on a study of the diarrhea problem from 1944 to 1951. During this time a total of 975 patients were under observation. Particular attention was given to a study of the stool cultures for enteric organisms and to a critical evaluation of the effectiveness of water and electrolyte therapy. Selected chemotherapeutic and antibiotic agents were tested therapeutically and *in vitro* with organisms isolated from patients with diarrhea. The following observations were made:

1. Diarrhea, enteritis and dysentery is a major cause of death in Texas. During an eight year period, 1941–48, they constituted over three times the expected deaths in children less than two years of age.

2. Rectal swab cultures from 111 infants with diarrhea and 186 control subjects of a similar age revealed remarkably little difference in the bacterial flora although *Salmonella* and *Shigella* were found somewhat more frequently in the older infants with

diarrhea. It was not possible to evaluate the significance of *Paracolobactrum*, *Proteus*, *Alcaligenes* and *Pseudomonas* as causative organisms in diarrhea.

3. Study of 2329 rectal swab cultures from 459 control subjects and 615 patients with diarrhea, mostly infants, disclosed a greater incidence of *Salmonella* and *Shigella* isolations in San Antonio than in Galveston.

4. *In vitro* resistance studies to neomycin, aureomycin, chloromycetin and streptomycin with 105 strains of enteric bacilli isolated from 85 patients with diarrhea disclosed that none of these antibiotics has a wide range of effectiveness. Aureomycin had the greatest effect in suppressing the growth of the majority of the ten types of organisms tested.

5. Rectal swab cultures from 25 diarrheal patients given neomycin showed that *Proteus* tended to be eliminated from the intestinal tract but that *Shigella* remained in pure culture.

6. There was no difference in the severity or length of the attack of diarrhea in 31 infants given neomycin therapeutically compared with 25 patients not given neomycin.

7. Administration of various sulfonamides to 64 patients with diarrhea did not seem to affect the course of the disease significantly compared with 58 subjects similarly treated except for the administration of a sulfonamide. Also by the alternate case method no marked difference in the duration of the diarrhea was noted when the results for 18 patients who received sulfathalidine were compared with 18 patients who did not receive sulfathalidine.

8. p-amino-phenyl-(1-phenyl-2-benzoyl-ethyl)-sulfone, an experimental drug known as Nu 404 was given to 66 infants with diarrhea. Although no differences in duration of the disease were noted between the infants who received Nu 404 and 84 control subjects who did not receive the drug the clinical impression was that the drug had sufficient merit in seriously ill patients to warrant further study.

9. An effective regimen directed toward the prevention of severe dehydration and acidosis was carried out on an outpatient basis for 120 infants mildly to severely ill with diarrhea in an area with high infantile diarrhea death rates.

10. Use of the regime developed by Darrow was effective in the treatment of 71 hospitalized infants with diarrhea.

11. Over a period of 3½ months only six deaths (two within ½ hour of admission) occurred amongst 182 infants with diarrhea at a time of the year and in a part of the country with a high infant death rate when the above integrated program of treatment was carried out. The higher death rates from diarrhea both before and after the 3½ month study further point the way to the need for a maintained program.

12. The physician plays the determining role in whether or not the infant succumbs when afflicted with diarrhea. He must have clinical facilities and skilled professional assistance to carry out his program. There is no need for death rates in Texas infants to be high.

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THE EFFECT OF HYPO- AND HYPERTHYROIDISM ON THE PITUITARY-ADRENAL RESPONSE TO STRESS*

MELVIN HESS† AND JOHN C. FINERTY

The pituitary-adrenal mechanism is closely associated with thyroid function as shown by clinical as well as animal experimental data. Balze *et al.* (1951) demonstrated an inhibition of the adrenalinic eosinopenia following thiouracil treatment of hyperthyroid and normal patients. Patients with Addison's disease show a ten-fold greater incidence of hyperthyroidism than the normal population (Frederickson, 1951). In addition, Frederickson reported complete suppression of thyroid function with large doses of cortisone. Both adrenocorticotrophic hormone (ACTH) and cortisone inhibit the increase in protein-bound iodine which follows administration of thyroid-stimulating hormone (TSH) (Woodbury, Ghosh and Sayers, 1951). Daughaday and Farr (1951) reported abnormal adrenocortical function in severe hyperthyroid patients. They showed that such patients fail to exhibit the normal rise in 17-ketosteroids after ACTH injections; and that the average fall in blood eosinophils was 46 per cent compared to a normal fall of 76 per cent four hours after administration of 25 mg. of ACTH. They suggest that severe hyperthyroidism is a stimulus for increased adrenal activity which may result in decreased adrenal reserve.

Since thyroid deficiency is believed to cause adrenal cortical atrophy and at the same time induces profound changes in the cytological structure of the anterior hypophysis, it should provide an experimental condition for correlating pituitary cellular structure with adrenotrophic function.

The adrenal cortical atrophy found by many workers after relatively long periods (6 weeks or more) of thyroid deficiency (induced by goitrogenic drugs or surgically) has usually been

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attributed to reduced ACTH secretion. However, this conclusion has been based upon inductive reasoning rather than upon experimental evidence. Some investigators deny that adrenal atrophy accompanies thyroid deficiency, especially since in many cases adrenal weight loss is not proportionately greater than body weight loss; but Zarrow and Money (1949) have shown that after 6 weeks or more there is a relative as well as an absolute loss of adrenal weight. Deane and Greep (1947) demonstrated that the atrophy occurs in the zona fasciculata of the adrenal cortex, whereas this zone undergoes hypertrophy in hyperthyroidism. These authors attribute the hypertrophy to an increased secretion of ACTH, which is supported by the work of Wallach and Reineke (1949) who were able to inhibit the increased adrenal weight of hyperthyroidism with adrenocortical extract.

Complete loss of pituitary acidophilic granules and increase in percentage of basophile cells has been well established by Griesbach and Purves (1945) and others following complete thyroid deficiency. If two types of basophiles are differentiated, the beta cells (of Halmi) which are apparently identical to the "thyrotrophs" (of Purves and Griesbach) increase in number and size following thyroidectomy (Halmi, 1952; Purves and Griesbach, 1951).

When considering the role of thyroid deficiency in the pituitary-adrenal mechanism, certain questions immediately arise:

1) Does thyroid deficiency alter the sensitivity of the adrenal to ACTH stimulation?

Zarrow and Zarrow (1951) have shown that the atrophic adrenal of a thiouracil-treated rat is just as responsive to ACTH as a normal gland, and suggested decreased ACTH secretion as the cause of adrenal atrophy.

2) Does thyroid deficiency change the rate of production or storage of ACTH in the pituitary?

Halmi and Bogdanove (1951) found no significant differences in ACTH content of intact and thyroidectomized (30 days) rat pituitary glands, and suggest failure of ACTH release as cause of adrenal atrophy.

3) Is failure of ACTH release from the pituitary the cause of adrenal atrophy?

Gabrilove and Soffer (1950) demonstrated that rats treated with propylthiouracil react normally to ACTH and epinephrine as demonstrated by adrenal ascorbic acid content in spite of involution of the cortex and decreased resting content of Vitamin C.

These same questions may be applied to the role of thyroid excess in the pituitary-adrenal mechanism. Does thyroid excess alter the sensitivity of the adrenal to ACTH stimulation or change the rate of production or storage of ACTH in the pituitary? Is increased release of ACTH from the pituitary the cause of adrenal hypertrophy?

The following series of experiments were designed to test these points using different methods of stress after surgical thyroidectomy or thyroid feeding.

Methods

Male rats of the Holtzman strain were used throughout this study. Some rats were made thyroid deficient by total thyroid-parathyroidectomy, and maintained on Purina Laboratory Chow pellets. To induce a hyperthyroid condition in others, Armour thyroid powder was mixed with ground chow in a concentration of 0.5 per cent by weight, and fed to intact rats. All animals were permitted to take food and tap water *ad libitum*.

As a means of stress, on one occasion 0.5 mg. of histamine per 100 gm. of body weight was injected intraperitoneally. Otherwise, the rats were anesthetized with nembutal and stressed by scalding—by immersing the animal (except the head) in water at 70 degrees C. for 5 seconds.

The pituitary glands utilized for study of their cytological composition were fixed in a Zenker-formol solution, sectioned at 4 micra and stained with a modified azocarmine stain (Briseno and Finerty, 1949).

The analysis of the adrenal glands for their ascorbic acid content was determined by the method of Roe and Kuether (1943).

The determination of the ACTH content of the pituitary glands of control, hypo- and hyperthyroid animals (44–48 days of thyroid deficiency or excess) was undertaken in the following

manner: The pituitaries of rats from each group were removed, posterior lobes discarded, the remainder weighed on a torsion balance and placed in an homogenizing tube immersed in an ice-water bath. The glands from each group of animals were pooled and then ground with sufficient 0.9 per cent NaCl, made acid to 0.1N with HCl, to make a concentration of 40 mg. of tissue per 1 cc. The tube containing the homogenate was immersed in a boiling water bath for one hour. The homogenate was stored in the frozen state. At the time of assay, the homogenates of pituitary tissue were further diluted with 0.9 per cent NaCl and 0.9 per cent NaCl made acid to 0.01N with HCl, so that 2 mg. of tissue were contained in 1 cc. at 0.01N HCl. Young male rats, hypophysectomized 24 hours previously, were used for the assay. After removal of the left adrenal gland under nembutal anesthesia, 0.5 mg. of pituitary tissue per 100 gm. of body weight was administered to each rat via the tail vein. At the end of one hour, the rat was sacrificed and the right adrenal gland removed. The difference in the ascorbic acid content of the right adrenal gland from that of the left gland was taken as an index of the ACTH activity.

Results

Experiment I: In a preliminary experiment, six-week-old male rats were thyroidectomized and 21 days later were stressed by histamine injection. One hour following stress, the rats were decapitated and the ascorbic acid content of the adrenal glands determined. Intact histamine-injected rats, uninjected thyroidectomized rats and uninjected intact rats of the same age and sex served as controls. The pituitary glands were removed and quantitative analysis of the cell types was made.

As can be seen from Table I, 21 days of thyroid deficiency evoked no change in the body or adrenal weights as compared to that of controls, either before or after stress. The values for body and adrenal weights overlap from one group to the next. However, there is more than a two-fold increase in the per cent of adrenal ascorbic acid depletion in the thyroidectomized rats subjected to stress. The pituitary cell type analysis (Table II) shows a marked decrease in the per cent of acidophiles and increase in basophiles of thyroidectomized rats.

Experiment II: The experiment was repeated with a longer interval following thyroidectomy (36 days) and using a more severe stress—scalding. Scalding is a severe stress which causes a marked depletion of adrenal ascorbic acid, but there is no statistically significant difference in the percentage of pituitary cell types in the scalded rats as compared to the controls (Finerty, Hess and Binhammer, 1952).

TABLE 1

Effect of Stress on Thyroidectomized Rats

Treatment	No. of Rats	Body Wt. gm.	Adrenal Wt. mg.	Adrenal mg./100 gm. tissue	Ascorbic Acid % decrease
Untreated	3*	130 (95–155)‡	14.4 (12.6–17.8)‡	289 (260–319)‡	
Intact plus histamine injection† 1 hr. before autopsy	2*	158 (155–180)	12.6 (11.8–12.8)	225 (221–225)	22
Thyroidectomized for 21 days	2*	158 (155–160)	12.5 (12.4–12.6)	361 (360–364)	
Thyroidectomized for 21 days plus histamine injection† 1 hr. before autopsy	3*	143 (115–174)	11.2 (10.1–11.9)	192 (184–197)	47

*Each adrenal gland was analyzed separately for ascorbic acid content so that two determinations were made for each rat.
†0.5 mg. histamine/100 gm. body weight.
‡Range of figures.

When rats which have been thyroidectomized 36 days previously are compared to controls, a retardation of body growth is noted (Table III). Although the absolute adrenal weight of the thyroidectomized rats is lower than the controls, there is no difference if the glands are calculated on the basis of adrenal weight per 100 gm. of body weight. Following scalding, the ascorbic acid content of the adrenals of control rats is depleted by 28 per cent in one hour, whereas in thyroidectomized rats there is a 66 per cent depletion.

No change in the pituitary cellular picture occurs as a result of scalding (Table IV). In the thyroidectomized and thyroidectomized-scalded rats, there is a complete disappearance of pituitary acidophiles, with an increase in the number of basophiles of the type characteristic of thyroidectomy.

TABLE 2

Effect of Thyroidectomy and Stress on Pituitary Cytology

Treatment	No. of Rats	Pituitary Cell Analysis Acid. %	Baso. %	Chrom. %
Untreated	3	35.0 (28.2–41.7)†	6.7 (6.4–6.9)†	58.3 (51.4–65.4)†
Intact plus histamine injection* 1 hr. before autopsy	2	38.9 (34.4–43.4)	8.6 (6.6–10.5)	52.5 (46.1–59.0)
Thyroidectomized for 21 days.....	2	1.3 (0–2.6)	19.8 (18.6–20.9)	78.9 (76.5–81.4)
Thyroidectomized for 21 days plus histamine injection* 1 hr. before autopsy	3	3.3 (2.0–5.7)	13.2 (10.8–15.7)	83.5 (78.6–87.2)

*0.5 mg. histamine/100 gm. body weight.
†Range of figures.

Experiment III: Five-week-old male rats were thyroidectomized or fed thyroid powder for 44–48 days. One half of each group, including controls, was stressed by scalding. One hour following the scalding, the rats were sacrificed and their adrenals analyzed for ascorbic acid content. The pituitaries were prepared for histological study.

The adrenals of the thyroidectomized rats are equivalent in weight to those of the controls, if calculated on a body weight basis (Figs. 3 and 4). In the case of the thyroid-fed rats, on the other hand, there is a definite hypertrophy of the adrenal glands (Fig. 5). The stressed control rats showed a 38 per cent depletion of adrenal ascorbic acid, whereas the hypothyroid ones showed a 75 per cent and the hyperthyroid ones a 31 per cent depletion one hour after scalding (Table V).

The pituitary cell analysis of these rats, together with controls, is shown in Table VI. There is no statistically significant difference in the percentage of cell types or their appearance within each group whether or not they were stressed. The complete absence of acidophiles and increase in basophiles of the thyroidectomized animals were noted (Figs. 1 and 2). In the case of the animals receiving thyroid powder, a significant decrease in percentage of acidophiles of the non-stressed ones,

TABLE 3

Effect of Stress on Thyroidectomized Rats

Treatment	No. of Rats	Body Wt. gm.	Left Adrenal Wt. mg.	Left Adrenal Wt. mg./100 gm. body wt.	Adr. Ascorbic Acid mg./100 gm. tissue	Acid % drop
Untreated	5	240±19*	16.6±1.0*	7.0±0.4*	443±20*	
Intact plus scalding for 5 sec. @ 70°C.	5	260±15	19.1±0.6	7.4±0.5	317±13†	28
Thyroidectomized for 36 days	5	158±10§	12.1±0.9§	7.8±0.7	446±13	
Thyroidectomized for 36 days plus scalding for 5 sec. @ 70° C.	6	146± 7§	10.6±0.9§	7.8±0.8	149±22‡	66

*Standard error.
†Difference from untreated group statistically significant.
‡Difference from other three groups statistically significant.
§Difference from intact animals statistically significant.

TABLE 4

Effect of Thyroidectomy and Stress on Pituitary Cytology

Treatment	No. of Rats	Pituitary Cell Analysis Acid. %	Baso. %	Chrom. %
Untreated	5	39.6±0.8*	6.3±0.4*	54.1±0.9*
Intact plus scalding for 5 sec. @ 70° C.	5	41.4±1.7	6.5±0.2	52.1±1.8
Thyroidectomized for 36 days	5	0	21.6±3.5	78.4±3.5
Thyroidectomized for 36 days plus scalding for 5 sec. @ 70° C.	6	0	19.2±0.9	80.8±0.9

*Standard error.

in comparison to the untreated animals, was observed. The basophiles of the thyroid-fed group were similar in appearance and number to those of the untreated animals.

PLATE 1

FIG. 1. The pituitary gland of a control animal, stained with an azocarmine stain. The acidophilic granules appear dark, whereas the basophiles are the large light-colored cells. Only the nuclei of the chromophobes are visible. The pituitaries of animals in this group scalded one hour before autopsy had the same appearance and percentages of cell types. 700 X.

FIG. 2. The pituitary gland of a rat thyroidectomized for 46 days, stained with an azocarmine stain. There is a complete disappearance of acidophilic cells and the basophiles have increased in number and size, contain vacuoles and appear hyalinized. Scalding one hour before autopsy did not result in any cytological changes in the pituitaries of thyroidectomized rats. 700 X.

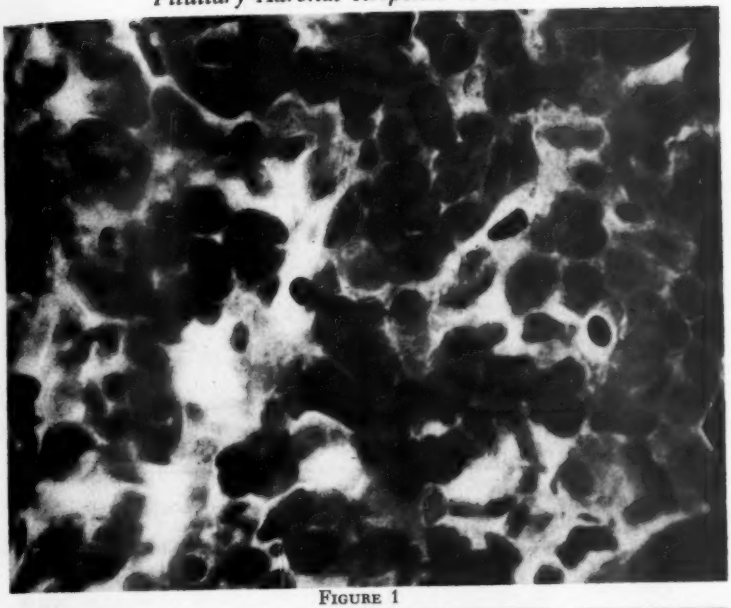

FIGURE 1

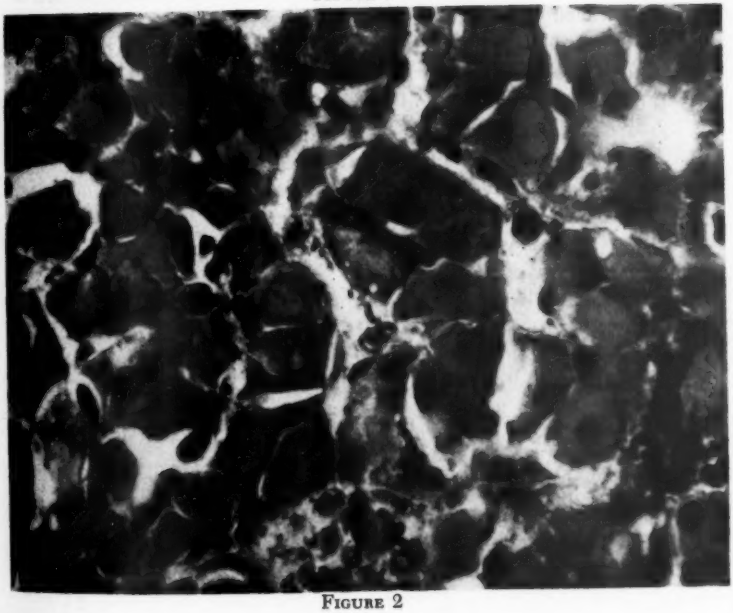

FIGURE 2

TABLE 5

Effect of Stress on Hypo- and Hyperthyroid Rats

Treatment	No. of Rats	Body Wt. gm.	Left Adrenal Wt. mg.	Left Adrenal Wt. mg./100 gm. body wt.	Adr. Ascorbic acid mg./100 gm. tissue
Control	10	274±8*	18.4±0.7*	6.7±0.2*	423±17*
Control plus scalding for 5 sec. @ 70° C.	10	247±8	17.5±1.0	7.0±0.2	263±19†
Thyroidectomized for 44–48 days	9	144±5	11.5±0.2	8.1±0.4	538±17
Thyroidectomized for 44–48 days plus scalding for 5 sec. @ 70° C.	9	134±3	10.1±0.4	7.6±0.4	135±41†
Thyroid-fed for 44–48 days	5	212±13‡	29.4±2.3‡	13.9±1.0‡	360±18
Thyroid-fed for 44–48 days plus scalding for 5 sec. @ 70° C.	6	224±11‡	24.9±0.8‡	11.3±0.8‡	247±13†

*Standard error.
†Difference from corresponding non-stressed group statistically significant.
‡Difference from controls statistically significant.

Experiment IV: The next experiment was undertaken to determine the sensitivity of the adrenal gland to an injection of a standard preparation of ACTH (Armour ACTHAR). Control, thyroidectomized and thyroid-fed rats were anesthetized with nembutal and the left adrenal gland removed. They were then injected via the tail vein with the equivalent of 5 micrograms of standard ACTH (LA-1A) per 100 gm. of body weight. After one hour, the animals were sacrificed and the right adrenal removed. The ascorbic acid content of both adrenals was determined, and the difference between the right and left glands taken as an index of ACTH stimulation. The control animals showed a depletion of adrenal ascorbic acid of 30 per cent and the hyperthyroid rats a similar response (27 per cent).

PLATE 2

FIG. 3. The adrenal cortex of a control rat, stained with Sudan IV. The sudanophilic lipid materials are present in the zona glomerulosa and zona fasciculata, with a lipid-poor transitional zone between. Scalding one hour before autopsy evoked no changes. 130 X.

FIG. 4. The adrenal cortex of a rat thyroidectomized for 46 days, stained with Sudan IV. The zona fasciculata is somewhat narrower than in controls, with the fat restricted almost entirely to the outer part of this zone. The fat stains a much deeper red in these glands than in those of controls. No difference in the appearance of the adrenal cortex was noted after scalding one hour before autopsy. 130 X.

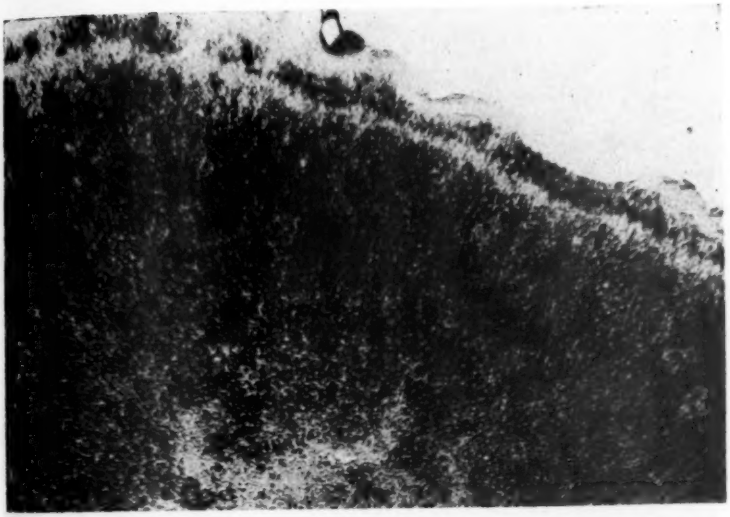

FIGURE 3

FIGURE 4

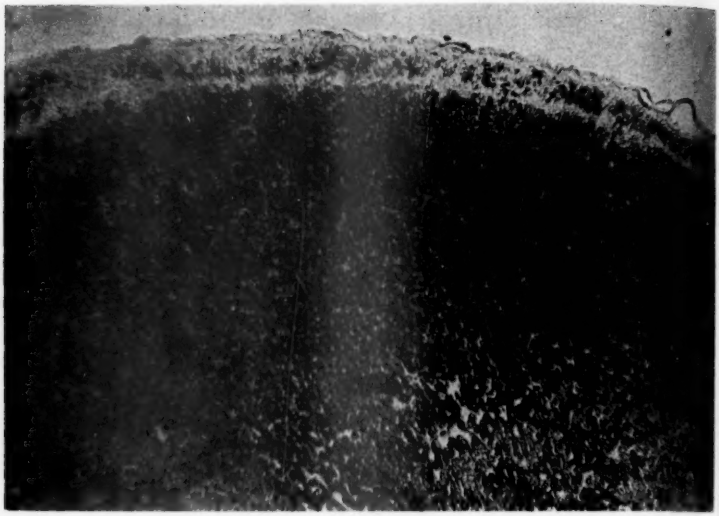

FIGURE 5

PLATE 2 *(Continued)*

FIG. 5. The adrenal cortex of a rat fed thyroid powder for 46 days, stained with Sudan IV. The zona fasciculata is broader than in controls and the sudanophilic lipids appear slightly reduced in quantity The adrenal cortex of rats from this group which were scalded one hour before autopsy had the same appearance. 130 X.

However, hypothyroid rats showed a 48 per cent depletion of adrenal ascorbic acid after the ACTH administration (Table VII).

Experiment V: Analysis of the pituitary glands of control, hypo- and hyperthyroid animals for ACTH content revealed no significant differences. When injected in amounts of 0.5 mg. per 100 gm. of body weight into hypophysectomized rats, pituitary tissue from controls produced an adrenal ascorbic acid depletion of 22 per cent (in 11 rats); from thyroidectomized animals a depletion of 21 per cent (in 10 rats); and from thyroid-fed rats a depletion of 25 per cent (in 10 rats).

TABLE 6

Effect of Hypo-, Hyperthyroidism and Stress on Pituitary Cytology

Treatment	No. of Rats	Pituitary Cell Analysis Acid. %	Baso. %	Chrom. %
Control	10	40.4±1.5*	7.0±0.2*	52.6±1.5*
Control plus scalding for 5 sec. @ 70° C.	14	37.2±1.2	7.4±0.4	55.1±1.4
Thyroidectomized for 44–48 days	9	0	25.8±1.2	74.2±1.2
Thyroidectomized for 44–48 days plus scalding for 5 sec. @ 70° C.	13	0	25.3±1.0	74.7±1.0
Thyroid-fed for 44–48 days	5	31.0±2.6	6.4±0.6	62.5±3.0
Thyroid-fed for 44–48 days plus scalding for 5 sec. @ 70° C.	6	35.6±2.3	6.5±0.4	57.9±2.4

*Standard error.

TABLE 7

Adrenal Sensitivity Test

Treatment	No. of Rats	Adrenal Ascorbic Acid mg./100 gm. tissue Before injection	After injection†	% decrease
Control	5	442±26*	309±24‡	30
Thyroidectomized for 44–48 days	5	355±45	183±20‡	48
Thyroid-fed for 44–48 days	5	376±12	274±16‡	27

*Standard error.
†5 micrograms ACTH/100 gm. body wt.
‡Difference from groups before injection statistically significant.

Discussion

The rats used in this study were young growing animals in which the thyroid gland is essential for normal growth (Means, 1948). In the case of the rats which were thyroidectomized for 21 days, no retardation of body growth was noted. This can be explained by the fact that the absence of the thyroid gland for this period was not sufficient to inhibit growth, since thyroid hormone circulates and is effective in the body for a considerable length of time (Means, 1948). Animals which had been thyroidectomized for 36 days and for 44–48 days exhibited the

retardation of body growth which usually follows removal of the thyroid gland in growing animals after a period of time.

In this study no adrenal atrophy was noted following thyroidectomy if the glands were calculated on the basis of body weight. Since body growth was retarded in rats which were thyroidectomized for longer periods of time, the failure to note the adrenal atrophy of hypothyroidism cited by many previous investigators may be a reflection of the reduced body weight. Experiments should be conducted utilizing adult, fully grown rats, so that adrenal weight studies following thyroidectomy would not be influenced by differences in the body weights of control and thyroidectomized animals. The adrenal hypertrophy observed in hyperthyroidism is in accord with the results of other workers.

Further evidence of the severity of scalding as a means of stress can be found in the report by Finerty, Hess and Binhammer (1952), in which they observed a pronounced adrenal enlargement 24 hours after rats had been scalded in the manner utilized in this study.

It may be noted, especially in the 44–48 day hypo- and hyperthyroid rats (Table V), that before stress the adrenal ascorbic acid value of the thyroidectomized rats is greater and that of the thyroid-fed rats less than the control group. Since Daughaday and Farr (1951) suggest that hyperthyroidism is a stimulus for increased adrenal activity, it is not surprising that the resting level of ascorbic acid in the adrenal should be lower than that found in control animals. Using a similar line of reasoning, the increased resting level of adrenal ascorbic acid found in hypothyroid rats may be due to the reduced metabolic rate and decreased adrenal activity.

It is apparent that the adrenal glands of hypothyroid rats are more sensitive to both ACTH administration and severe stress than those of intact or hyperthyroid rats. The increased adrenal sensitivity in hypothyroidism is also evident from the lower-than-normal adrenal ascorbic acid value of such rats even before administration of the ACTH. This can probably be explained by the fact that the nembutal anesthesia was capable of stimulating a depletion of adrenal ascorbic acid in these hypersensitive, non-hypophysectomized rats.

The more marked depletion of adrenal ascorbic acid in hypothyroidism may be a result of a change in pituitary ACTH content, delayed inactivation of circulating ACTH, or an intrinsic difference in adrenal gland responsiveness. The results of Halmi and Bogdanove (1951), as well as of this study, show no difference in the pituitary ACTH content. Hypothyroidism may result in a prolonged time necessary for the inactivation of ACTH, thereby enabling this hormone to act on the adrenal cortex over a longer period of time. It is also possible that a reduced rate of synthesis of vitamin C in hypothyroid rats may aid in explaining the low adrenal ascorbic acid values after stress. It might be a combination of both the delayed inactivation of ACTH by the body and an increased adrenal sensitivity in hypothyroidism, together with problems of synthesis, to account for the greater depletion of adrenal ascorbic acid seen after subjecting thyroidectomized rats to severe stress.

The increased target organ sensitivity of hypothyroid animals is also reflected in thyroid-gonadal studies. Schockaert (1931) has shown that the enlargement of secondary sex organs in male rats given anterior pituitary injections is significantly increased by thyroidectomy. The greatest gonadal response secured by Smith and Engle (1930) from pituitary implants was in a thyroidectomized animal.

Although the ACTH content of the pituitaries of thyroid-fed rats did not differ from that of control animals, the enlarged adrenal gland found in hyperthyroidism might still be attributed to an increased ACTH secretion. Wallach and Reineke (1949) were able to prevent the adrenal enlargement of hyperthyroidism with the administration of adrenocortical extract, which would inhibit the increased rate of formation of ACTH in the pituitary. It is entirely possible that there can be an increased rate of formation and release of ACTH from the pituitary without an accompanying increased storage of the trophic hormone. The assay procedure utilized in this study would only determine stored ACTH in the pituitary.

The cytological structure of the pituitaries of hypothyroid rats was like that observed previously by Griesbach and Purves (1945) and others. That the rats were totally thyroidectomized is evident by the complete degranulation of the acidophiles. Goldberg and Chaikoff (1952) reported that 0.05 to 0.10 micro-

grams of thyroxine per 100 gm. of body weight per day would effectively prevent total acidophilic degranulation in the thyroidectomized rat, so that absence of acidophiles is a very sensitive test for completeness of the operation.

The cytological study of pituitary glands of thyroid-fed rats did not reveal the same findings that were cited by Severinghaus, Smelser and Clark (1934). The dosage of thyroid powder fed to the rats was sufficient to induce a hyperthyroidism, since there was a loss of body weight in the thyroid-fed rats in comparison to the controls (Table V), and a number of animals in the former group died, presumably from thyrotoxicosis. In addition, the rats receiving thyroid powder had an increased oxygen consumption, about 30 per cent higher per 100 gm. of body weight per hour, than that of control animals. The increase in size and number of the basophiles and the presence of numerous "castration" cells cited by the aforementioned authors were not noticed on examination of pituitaries of hyperthyroid rats. Instead, the basophiles appeared normal and in a percentage similar to control animals. Likewise, the acidophiles were no larger or more prominent than those observed in the pituitaries of control animals. However, a statistically significant reduction in the number of acidophilic cells was noted in the thyroid-fed group, which was not present after animals from this group were stressed. On the whole, the pituitaries of the thyroid-fed animals could not be differentiated from the controls by microscopic examination.

Summary

Even though a profound change occurs in the cytological structure of the hypophysis as a result of thyroid deficiency, the pituitary-adrenal mechanism responds to severe stress, as indicated by adrenal ascorbic acid, with depletions greater than under normal conditions. The response of the adrenal in hyperthyroidism parallels that of control animals.

Following thyroidectomy all acidophilic granules disappear from the hypophysis and certain basophilic cells increase two to three hundred per cent. The pituitary cytological picture in hyperthyroidism resembles the normal.

The adrenal glands of the thyroidectomized rats are smaller than controls, and those of thyroid-fed animals are larger. However, since the hypothyroid rats have a greatly reduced body weight, their adrenals are similar to intact rats when calculated on a relative basis.

The adrenals of thyroidectomized rats are more sensitive to ACTH administration whereas those of hyperthyroid rats do not differ from controls.

No difference in the ACTH content of pituitaries of control, hypo- and hyperthyroid rats could be detected.

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MECHANISMS OF RESISTANCE AND DEPENDENCE IN GROWTH OF LEUKEMIC CELLS[1, 2]

L. W. Law

The eventual failure to achieve remissions with anti-leukemic substances, particularly folic acid analogs, in patients with acute lymphocytic leukemia has been reported on several occasions. Thus, it is indicated, but not proven, that resistance to folic antagonist is an explanation for the eventual failure to respond. This phenomenon is a problem of major concern in the treatment of the leukemic patient.

The use of antimetabolites, particularly the folic acid analogs, in the clinic and in the laboratory, are of special interest for the following reasons: 1) they are the most effective of known anti-leukemic agents, producing in leukemic mice increases in survival time as great as 200 percent, 2) in some instances selectivity of action against leukemic cells is striking, and 3) the inhibitory action of folic analogs in acute lymphocytic leukemia can be shown to be of a competitive, or at least semi-competitive nature. This affords a better means of elucidating the metabolic reactions involved in the therapeutic experiments.

The material presented here concerns the experimental production of transformations in leukemic cells of the mouse, the biologic characterization of the variant cells, investigation of the manner of origin of resistance and dependence and a consideration of the mechanisms involved in these phenomena.

The development of resistance and dependence in mouse leukemic cells, derived from a transplantable, acute, lymphocytic leukemia, designated L 1210 (Law, L. W., *et al.*, 1949) has been reported (Law, L. W. *et al.*, 1950, 1951). Three distinct transfer lines were developed by consecutive serial transfer of sensitive leukemic cells in mice receiving the M.T.D. of 3 different folic antagonists: 4-amino, N^{10}-methyl PGA (A-me-

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[1]National Cancer Institute, Public Health Service, Federal Security Agency, Bethesda, Maryland.

[2]Technical assistance given by P. J. Boyle and J. H. Miller is gratefully acknowledged.

thopterin), 4-amino, 9-methyl PGA (A-ninopterin) and 4-amino, 9, N^{10}-dimethyl PGA (A-denopterin).[3] See text figure No. 1. Sublines AD-R and AN-R were developed from the 60th transfer of the sensitive, control leukemia L 1210 by consecutive

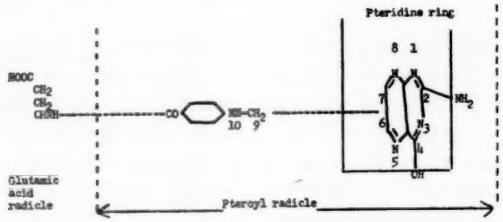

FIG. 1. Molecule of PGA. The folic analogs used in the present study were: 4-amino-PGA (aminopterin) which has an NH_2 group at the 4 position of the pteridine ring; 4-amino-9-methyl-PGA (A-ninopterin), which in addition to the 4-NH_2 substituent has a CH_3 group at position 9 in the pteroyl radicle; 4-amino-N^{10}-methyl PGA (A-methopterin), like A-ninopterin but with the CH_3 at position 10: 4-amino-9,N^{10}-dimethyl-PGA (A-denopterin) with CH_3 substitutions at both 9 and 10 positions; 4-amino-PAA (Amino-an-fol) with the NH_2 group at the 4 position of the pteridine ring and an aspartic acid radicle in place of glutamic acid; 9-methyl PGA, a weak antagonist having only a CH_3 substitution at the 9 position and N^{10}-methyl PGA having a CH_3 group at position 10 of the pteroyl radicle (see Jukes, Franklin, and Stokstad, 1950, for a discussion of these compounds).

serial transfers, subcutaneously, in mice receiving 3 mg. per kilogram body weight, of the folic antagonists A-denopterin and A-ninopterin, respectively, for 10 days or longer. Transfer of leukemic cells in a standard dose in Lockes' solution were always accomplished, as soon as possible when the localized lymphomatous growth was large enough to transfer in a brei-suspension (Law, L. W., and Boyle, P. J., 1950). Subline AM-D was developed in a similar manner from the 65th transfer of the sensitive control leukemia (see text figure 2).

The AM-D subline, dependent upon A-methopterin for optimal growth, has now been carried through 86 consecutive transfers, 80 since emergence of dependence. The AN-R subline, resistant to A-ninopterin, was carried through 36 consecutive transfers in mice receiving folic antagonist and then discontinued. These lines continued as stable lines and no rever-

[3]All folic analogs were kindly supplied by the Lederle Laboratories Division, American Cyanamid Co., Pearl River, New York.

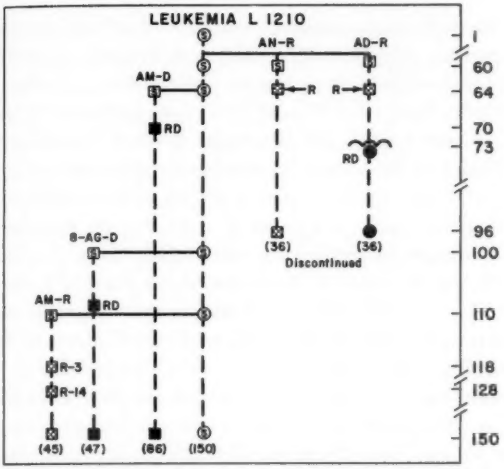

FIG. 2. Showing origin of 5 variant sublines from sensitive leukemia L 1210:AN-R (resistant to A-ninopterin), AD-R (resistant to A-denopterin), AM-D (dependent on A-methopterin), 8 AG-D (dependent on 8-Azaguanine) and AM-R (resistant to A-methopterin). S represents cells sensitive to folic analogs and 8-Azaguanine; R represents resistance and D dependence. Squares represent serial transfer of leukemic cells in host animals receiving antagonist; circles represent transfers in antagonist-free mice. Cross-hatching represents resistant cells; block figures, dependent cells. Waved line at transfer generation 73 in AD-R subline represents subsequent transfer in antagonist-free hosts. Figures in parenthesis at bottom of each transfer line represent present transfer generation.

sions to sensitivity have occurred. At the 73rd transfer of leukemic cells in the AD-R subline, dependent on A-denopterin for optimal growth, transfers were made in mice not given antagonist and continued in serial transfer for 23 consecutive generations in the absence of antagonist (Text fig. 2). During this period the variant cells has remained resistant to and dependent upon this specific folic antagonist, when tested at various transfer periods. Resistance to A-denopterin, in this specific subline, may, therefore, be considered a stable and irreversible heritable change.

Substances Inhibiting and Stimulating Growth of AM-D Cells

The possibility was considered that resistance developed to a folic acid antagonist might extend to other agents exerting

specific anti-leukemic effects. The effect of such agents was studied on the AM-D subline, dependent upon A-methopterin for optimal growth. 8-Azaguanine (guanazolo) (Kidder, G. W., *et al.*, 1949; Law, L. W., 1950) alpha-peltatin (Greenspan, E. M., *et al.*, 1950) 2,6 diaminopurine (Burchenal, J. H., *et al.*, 1949), Compound E, cortisone (Heilman, F. R., and Kendall, E. C., 1944) and 2,4,6-triethyleneimino-s-triazine (TEM) (Burchenal, J. H., *et al.*, 1950), all of which show leukemia-inhibiting properties, were used at the approximate MTDs shown in table 1. Transfer generations 77 through 87 of the AM-D subline were used to test the effect of anti-leukemic agents and corresponding transfer generations of the sensitive L 1210 subline were used as control material.

Cortisone and 2,6 diaminopurine did not produce a significant inhibition of growth of leukemic cells in the sensitive control line nor in the A-methopterin-dependent line and are not included in the tabular material. It can be seen by reference to table 1 that the degree of inhibition produced by 8-Azaguanine and alpha-peltatin is nearly identical in the sensitive and dependent sublines whereas TEM is a more effective inhibitor of the dependent cell growth. In contrast to the effects obtained by unrelated anti-leukemic agents on the growth of sensitive and dependent leukemic cells, an unequivocal cross-resistance was obtained when AM-D leukemic cells were grown in mice receiving four closely related antifolic compounds: A-ninopterin, A-denopterin, Amino-an-fol (4-amino PAA) and, amino-

TABLE 1

Comparative sensitivity of A-methopterin-dependent (AM–D) and sensitive leukemic cells (L 1210) to several anti-leukemic agents

Compound	Dosage (mg/kg) Daily	Total	Sensitive	Dependent
			Inhibition index*	
8-Azaguanine	50	400	0.13	0.17
Alpha-peltatin	5.0	15	0.06	0.06
TEM	0.75	3.0	0.27	0.08
A-ninopterin	3	12	0.04	0.94
A-denopterin	3	12	0.01	1.03
Amino-an-fol	30	120	0.20	0.78
Aminopterin	0.2	0.8	0.01	1.0

*Inhibition index = $\frac{\text{Mean wt. tumor-treated}}{\text{Mean wt. tumor-controls}}$

opterin (4-amino PGA). Nearly complete inhibition of growth was obtained at 9 days in the sensitive subline while optimal growth was obtained in the dependent (AM-D) line. No discernible effect of the parent compound, folic acid (PGA) or of Citrovorum factor (CF) was observed in either the sensitive or dependent subline. Optimal growth was obtained at levels of PGA as high as 30 mg./kg.$\times$ 4 and of CF at levels of 200,000 units $\times$ 5 (see also table 4). Burchenal *et al.* (1951) observed inhibition in growth of their Ak-sensitive and Ak-resistant leukemic lines when weak folic antagonists, particularly, 9-methyl PGA was incorporated in the diet. 9-methyl PGA and N^{10}-methyl PGA were ineffective in inhibting the growth of sensitive cells of leukemia L 1210 but did provide for nearly 50% optimal growth of dependent cells of the AM-D subline.

Growth Characteristics of AM-D Cells, Dependent Upon A-methopterin for Optimal Growth

An extensive analysis of the dependency characteristic in the AM-D subline is given in table 2. A striking increase in mean lymphomatous weight occurs in mice bearing AM-D leukemic cells and given A-methopterin parenterally, while growth of these leukemic cells in mice deprived of antagonist is not optimal, the final mean weight attained at 9 days being only one-half to one-fourth that in the treated group. On the other hand, nearly complete inhibition of growth of the sensitive line is achieved at a total dosage of 12 mg./kg. body weight of A-methopterin and complete inhibition of growth at 9 days at dosage ranges from 20 to 48 mg./kg.

It is to be noted, in table 2, that the mean weight of dependent lymphomatous tissue, attained at 9 days, has doubled approximately during the course of transfer through 88 generations in DBA mice receiving folic antagonist. This probably represents a selection of dependent cells during this period.

Growth rates of the AM-D variant cells and of the control, sensitive leukemic cells following transplantation of the 102nd transfer of each subline are compared in text-figure 3. The growth rates of sensitive leukemic cells grown in untreated DBA mice (Curve I) and dependent cells grown in mice given 12 mg./kg. of A-methopterin (Curve III) are similar. The

TABLE 2

Dependence in leukemic cells of the AM–D subline of leukemia L 1210

	Transfer Generation*	No. Mice	A-methopterin Dosage (mg/kg) Indiv.	Total	Mean Lymphomatous Weight mg.	Dependency Ratio
Dependent	8–18	61	3	12	480.7±26.5	2.37†
		60	None		202.1±17.9	
	27–39	20	12	48	685.5	
		78	3	12	833.3	3.92
		29	None		212.7	
	43–96	49	3	12	985.3	2.45
		49	None		402.7	
Sensitive	73–85	43	3	12	10.3	
		47	None		732 ±41.4	
	98–108	20	12	48	0	
		30	3	12	6.0	
		55	None		1,190.0	
	127–157	28	3	12	10.9	
		28	None		805.4	

*The AM-D subline was developed from the 65th transfer of the sensitive, control line, thus transfer 8 of the dependent subline corresponds to transfer 73 of the control subline.

†Dependency Ratio: $\frac{\text{Mean lymphomatous weight, A-methopterin-treated mice.}}{\text{Mean lymphomatous weight, Antagonist-free mice.}}$

mean tumor volume attained at 10 days of AM-D leukemic cells, grown in antagonist-free mice, was only 0.84 cc. (Curve II), compared with a mean tumor volume 94.3 cc. when grown in mice given antagonist. Complete inhibition of growth of leukemic cells of the sensitive subline, grown in mice given 12 mg./kg. of A-methopterin, was observed in this transfer (Curve IV).

Response of Dependent Cells to Increases in Dosage of Antagonist

The morphology and biologic behavior of the sensitive subline of leukemia L 1210 has been closely observed. Terminally, following subcutaneous transfer of this leukemia, numerous leukemic cells are dispersed in lymph nodes, spleen and liver, enlarging these organs and obscuring the architecture. Leukemic cells appear in the blood a few days after transfer giving

TABLE 3

*Comparison in response to A-methopterin of dependent (AM–D) and sensitive, L 1210, leukemic cells**

Transfer line	Dosage (mg/kg) Daily	Total	Mean tumor wt. mg	Survival days	Hb	Peripheral blood counts wbc	agran.	gran.	blasts	Organ infiltrations Lymph nodes	spleen	liver
Dependent	3	12	1,362.7	12.4±.33	10.1	47,760	11,940	32,299	2,821	+++	+++	++
	5	20	1,146		12	24,930	7,719	16,000	1,211	+++	+++	++
	7.5	30	1,084		12	46,835	9,835	34,190	2,810	++	++	++
	12	48	685.7		13.2	6,685	1,872	4,783	30	++	+	—
Sensitive	0	0	1,478	12.7±.15	11.2	41,150	14,568	20,821	5,761	+++	+++	++
	3	12	0		13.5	11,025	6,550	4,520	55	—	—	—

*Mean tumor wt. and histologic data obtained on 10 mice in each group representing 100th transfer of resistant and sensitive cells in DBA mice; survival time determined on 20 mice in each group; blood studies represent means of 5 animals in each group.
+++ severe, ++ moderate, + slight,—no infiltration, of leukemic cells into designated organs as determined my microscopic study.

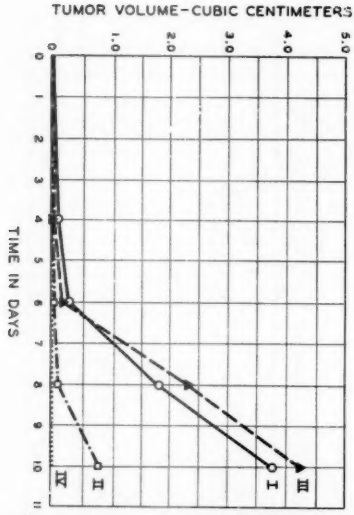

FIG. 3. Tumor volume measurements of AM-D (A-methopterin-dependent) leukemic cells of the 102nd transfer grown in DBA mice receiving A-methopterin (Curve III) and in antagonist-free mice (Curve II). 102nd transfer of sensitive leukemic cells shown in Curve I. Curve IV, sensitive L 1210 cells transferred to mice receiving 12 mg./kg. antagonist.

a moderately high leukocyte count. By reference to table 3 it can be seen that the behavior of the dependent leukemic cells grown in mice receiving A-methopterin (12 mg./kg.) is similar to the growth behavior of the sensitive cells grown in antagonist-free mice. There results a florid leukemia with massive lymphomatous growth subcutaneously, a striking secondary anemia, leukocytosis with escape of lymphoblasts into the peripheral blood, severe infiltration into subcutaneous and internal lymph nodes, spleen and liver and a mean survival time similar to that observed in untreated mice growing the sensitive leukemic cells. In contrast, note the nearly complete inhibition of leukemia in mice bearing the sensitive subline and given A-methopterin. It was of interest, therefore, to observe the effects of increasing dosage levels up to and including the LD_{50} on the growth response of the dependent leukemic cells. It can be seen that at levels of 5.0 and 7.5 mg./kg. (total dose of 20 and 30 mg./kg.) profuse localized growth

and a generalized leukemia result. At the dosage level of 12.0 mg./kg. (total dose 48 mg./kg.) which represents the LD_{50} for non-leukemic strain DBA mice and also of mice bearing the sensitive transplant, a reduction of localized growth to approximately 50% has occurred but infiltration into lymph nodes and spleen was still obtained. No deaths attributed to drug toxicity occurred in this group. Thus, it is indicated that the dependent cells now show at least a 50-fold increase in tolerance to A-methopterin, since it requires approximately 0.25 mg./kg. (total dose, 1.0 mg./kg.) of this PGA-antagonist to inhibit the localized growth of the sensitive cells to a similar weight-level.

In addition to the development of a florid leukemia in A-methopterin-treated mice bearing transfers of dependent leukemic cells, it is apparent that the PGA antagonist supplied to these mice is being used directly as a growth factor by the resistant cells or is being converted for use. It has been found in mice bearing the sensitive leukemic cells and given folic antagonist at a dosage level of 3 mg. or 5 mg./kg. every other day for 4 days that various symptoms of vitamin deficiency appear. There results a slight anorexia indicated in a lowered average food intake of 2.0 g. per mouse per day and a slight loss in weight over a 7-day period (0.3 g. or 0.2%). Untreated mice, bearing sensitive leukemic cells during this period, gain an average of 2.5 g. (12.5%) and consume an average of 2.5 g. of food per mouse per day. In contrast, leukemic mice bearing the dependent leukemia and given PGA antagonist at this dosage level show an average gain in body weight of 2.1 g. (10.5%) and consume 2.2 g. of food. Other signs of PGA deficiency fail to appear in mice growing the dependent leukemic cells and supplied with A-methopterin at the 3 mg. or 5 mg./kg. level. The red pulp of the spleen resembles that seen in the normal animal (Weir, D. R., *et al.*, 1948) indicating active hematopoiesis. Numerous megakaryocytes and many foci of nucleated cells of the erythroid series are found throughout the pulp. On the other hand, in mice bearing transplants of the sensitive subline, and given PGA antagonist, there is definite indication of a deficiency state. The red pulp contains relatively fewer cells with very few megakaryocytes and

nucleated red cells. There occurs also a reduction in the number of polymorphonuclear leukocytes. The bone marrow of mice bearing sensitive leukemic cells and given chronic doses of A-methopterin reveals the following signs of PGA deficiency: an increase in cellularity, mostly of larger immature cells with many in mitoses and a decrease in megakaryocytes, nucleated red cells and cells of the granulocytic series. These changes are evident particularly at the higher dosage level. The picture appears nearly normal, however, in DBA mice given the same dosage of antagonist but bearing transplants of dependent cells, indicating that the PGA antagonist is used, directly or indirectly, in the profuse growth of the dependent leukemic cells.

Reversal of Inhibitory and Growth-Stimulating Effects of Folic Antagonist

Citrovorum factor (C.F.) has been shown to be effective in preventing the toxicity of PGA antagonist in *Leuconostoc citrovorum* (Sauberlich, H. E., 1949) and in mice (Broquist, H. P., *et al.*, 1950), and in reversing the anti-leukemic action of these antagonists in mice (Burchenal, J. H., *et al.*, 1950). Since it appears that C.F. on a weight basis, is more effective than PGA in reversing the effects of folic antagonists, it was of interest to study the effects of this factor on the growth of the resistant and sensitive lines of leukemia L 1210 and to determine the blocking effect of C.F. on (a) the anti-leukemic activity of A-methopterin on sensitive leukemic cells and (b) the optimal growth-promoting capacity of this antagonist on dependent leukemic cells.

Citrovorum factor was injected intraperitoneally in mice at a dosage level of 2×10^6 units 24 hours after inoculation subcutaneously of a standard dose of leukemic cells and then 30 minutes prior to injection of 2 mg./kg. of A-methopterin. It can be seen from table 4 that C.F. did not affect the growth of the dependent leukemic cells, nor was any effect noted on the growth of sensitive leukemic cells. C.F., however, was effective in partially blocking the utilization of the PGA antagonist by the dependent leukemic cells. Only 71% optimal growth of dependent cells was obtained when C.F. was given prior to

TABLE 4

*Citrovorum factor (C.F.) and the effects of A-methopterin on sensitive and dependent (AM–D) leukemic cells of leukemia L 1210.**

Experiment	No. mice	Dosage† Daily	Dosage† Total	Relative‡ mean weight	Absolute mean weight mg (range)	% reversal
				dependent (AM-D)		
A-methopterin	42	2	8	1	(758.5–1362.8)	—
A-methopterin + C.F. §	28	2 2 x 10^6	8 10 x 10^6	.71	(544.1–854.3)	42
C.F.	36	2 x 10^6	8 x 10^6	.35	(275 –580)	—
Controls	36	0	0	.31	(195 –320.9)	—
				sensitive		
A-methopterin	54	2	8	.03	(0 –100.8)	—
A-methopterin + C.F.	44	2 2 x 10^6	8 10 x 10^6	.28	(87 –428.3)	29
C.F.	30	2 x 10^6	8 x 10^6	.91	(1087 –1330.3)	—
Controls	50	0	0	1	(704 –1559.4)	—

*Transfer generations 98–102 inclusive. Strain DBA mice of both sexes, weighing 18–22 g.
†Dosage of A-methopterin in mg/kg; citrovorum factor dosage in units/kg.
‡For convenience, optimal growth in both groups considered as 1.0.
§Citrovorum factor inj. intrap. 24 hr. prior to first inj. of antagonist, then ½ hr. prior to antagonist at 2, 4, 5, and 8 days. Citrovorum factor had an activity for *L. citrovorum* of 3 x 10^6 units per cc.

A-methopterin. This represents a partial reversal of optimal effect of antagonist equal to 42%. A partial reversal of the anti-leukemic effect of the antagonist on sensitive leukemic cells was also found (table 4). The reversal of anti-leukemic effect by C.F., as measured by tumor weight, was considerably less than that reported by Burchenal, *et al.*, (1950), who used survival time as a criterion, despite the fact that a considerably higher dosage level of C.F. was used in this study.

Folic acid (PGA) was also found to reverse, partially, the inhibitory action of A-methopterin in the sensitive leukemia and reversed the growth-promoting effect of this analog in the dependent strain. The ratio of analog to metabolite (PGA) for maximum effect was found to be approximately 1:15 and the ratio was the same for sensitive or dependent cells regardless of which was the inhibiting substance, providing that folic acid was always given prior to administration of A-methopterin. This suggests that the same enzymatic reaction is involved in both cases.

Other Characteristics of Dependent Leukemic Cells

No changes in morphology, antigenicity or transplantability have been observed in the variant forms of resistant or dependent cells developed in leukemia L 1210. Leukemic cells of the

sensitive or of the transformed sublines, as observed in localized growth or in infiltrations into spleen, liver, lymph nodes or in the peripheral blood, are morphologically indistinguishable. Attempts to detect antigenic differences by complement-fixation tests have been unsuccessful. Leukemic cells of the sensitive, control subline grow progressively, causing death of the host only in strain DBA, subline 2, mice and in F_1 hybrids using strain DBA as one of the parents. No changes in the transplantability of the resistant sublines have been observed. These cells fail to grow in mice of the C_{57} black, C_{58}, Balb C, RIL and C3H strains. More critical tests to determine such changes by the use of F_2 hybrid or backcross animals have not been accomplished.

Possible Mechanisms of the Dependence Phenomenon

The transformation to resistance and to dependence described here has provided a unique and specific modification of a metabolic pathway for biochemical studies. Examples of resistant mutant bacteria (Miller, C. P., and Bohnhoff, M. J., 1947; Newcombe, H. B., and Nyholm, M. H., 1950), and *Neurospora* (Emerson, Sterling, 1947) requiring specific drugs for growth have been reported, but it is not uncommon for reversion to occur in these forms. Although growth responses do not prove that a substance found to be necessary for growth is actually used as a metabolite it is highly probable that this is so in the AM-D line of leukemia. Certain mechanisms of dependence of these transformed cells on the specific folic analog, A-methopterin, or on any of the 4-amino-substituted folic antagonists may be considered:

(1) The transformed leukemic cells have acquired the ability to convert PGA antagonist to PGA or Citrovorum factor by one of several methods, by deamination of the antagonist at the 4 position of the pteridine ring or by methylation or acetylation of the 4-amino group. This possibility seems unlikely since neither PGA nor C.F. provides for optimal growth of the dependent cells. Furthermore, it has been shown that C.F. actually blocks, partially, the use of the PGA antagonist by the dependent cells.

(2) The variant cells have acquired the ability to convert A-methopterin to a compound which they can use, for example, reduced-A-methopterin. The work of Skipper, H. E., *et al.* (1952), however, clearly indicates that

hydrogenated A-methopterin did not stimulate the growth of L 1210 dependent leukemic cells.

(3) The resistant variants have acquired the ability to synthesize their own PGA or C.F. This possibility also seems unlikely for the same reasons given in (1) above.

(4) The leukemic cells have an increased ability to detoxify the PGA antagonist. From the studies reported here, it is apparent that toxic effects of antagonist do not appear in mice growing the transformed leukemic cells because the antagonist is metabolized in supporting optimal growth. If this were a case of detoxification of antagonist, there would not be expected a differential in the growth capacities of resistant leukemic cells supplied with or lacking antagonist. That is, detoxification could be an explanation for the resistance but not for the dependence characteristic, or

(5) The transformed leukemic cells have acquired the ability to use A-methopertin without conversion to PGA or C.F., employing a different mechanism for the synthesis of nucleic acids than that suggested as occurring normally.

A scheme giving a summary of a possible mechanism involved is shown in text figure 4. The A-methopterin-dependent line is shown as differing from the sensitive line in having the folic analog, PGA^1, combine with an abnormal apoenzyme, $AP\text{-}E^1$, giving a substance CF^1, which is necessary in the synthesis of nucleic acids in the dependent cells. The presence of a less efficient, normal apoenzyme system in the dependent cell would

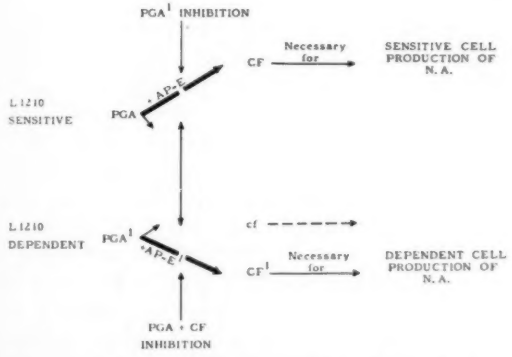

FIG. 4. Scheme showing possible interpretation of dependence-mechanism. Large arrows represent enzymatic reactions with breaks representing interruptions due to competitive inhibition. PGA = folic acid, PGA^1 = folic antagonist, C.F. = citrovorum factor, AP-E = normal apoenzyme, $AP\text{-}E^1$ = abnormal apoenzyme, NA= nucleic acids.

account for the sub-optimal growth of dependent cells in the absence of folic antagonist.

There are other interpretations of drug resistance and drug dependence which may fit this particular case especially the mechanism described by Skipper, H. E., *et al.* (1952) and by Hitchings (1952). Hitchings regards these phenomena as the result of a change in the geometry of the apoenzyme involved, resulting in a tighter binding of metabolite (PGA) to the enzyme and rejection of the antagonist (in this case A-methopterin). "Thus, in the dependent cells, it is assumed that this results in a 'super-normal activity' of the enzyme in question (when the analog is absent) and that this surplus activity in itself is inhibitory." The rôle of the drug, therefore, is to reduce the enzymic activity to the normal level and restore normal growth.

C-14 Labelled Na Formate Incorporation in Sensitive and Dependent Leukemic Cells

In an attempt to elucidate the mechanism involved, a study has been made of the incorporation of C-14 labelled sodium formate ($HC^{14}OO$ Na) into RNA and DNA purines of sensitive and dependent leukemic cells of leukemia L 1210 (Skipper, H. E., *et al.*, 1952). Folic acid antagonists appear to inhibit neoplastic growth by interfering with formate transfer which is essential to nucleic acid synthesis (Skipper, H. E., *et al.*, 1950). PGA at fairly high levels (60 mg./kg.) partially reverses this inhibition of formate incorporation into nucleic acids brought about by A-methopterin (Skipper, H. E., 1951).

It can be seen by reference to table 5, that specific differences are evident in the DNA and RNA turnover in sensitive and dependent tumor tissue and viscera of animals bearing these transplants as assayed in the isolated individual purines: 1) A-methopterin inhibits nucleic acid synthesis of the sensitive tumor mass and the viscera of animals bearing the tumor tissue. 2) Administration of A-methopterin approximately doubles the rate of DNA and RNA purine synthesis in the dependent tumor mass while profoundly inhibiting such synthesis in the viscera of these same animals. 3) The dependent tumor mass (without antagonist) does not incorporate formate

TABLE 5

Incorporation of C^{14}-formate into nucleic acid moieties of viscera and A-methopterin-sensitive and dependent (AM–D) tumor masses of leukemia L 1210

Group No.	Leukemia	Tissue	Treatment	Specific activities (μc/mole C)					
				Original Tissue	DNA Guanine	DNA Adenine	DNA Thymine	RNA Guanine	RNA Adenine
1	Sensitive	Viscera	None	6.9	171	280	...	199	248
	Sensitive	Tumor	None	4.2	206	227	100	309	308
2	Sensitive	Viscera	A-methopterin*	5.6	26	16	4	70	90
	Sensitive	Tumor	A-methopterin*	1.8	34	39	10	54	112
3	Dependent	Viscera	None	7.3	113	99	...	156	140
	Dependent	Tumor	None	1.8	55	51	24	85	86
4	Dependent	Viscera	A-methopterin†	4.9	15	10	3	37	35
	Dependent	Tumor	A-methopterin†	4.8	127	115	51	162	156

*A-methopterin (3 mg/kg) immediately before $HC^{14}OONa$ injection on seventh post-inoculation days.
†A-methopterin (3 mg/kg) on 3rd, 5th, and 7th days. $HC^{14}OONa$ on 7th day. (Data of Skipper, H. E., L. L. Bennett, Jr. and L. W. Law.)

as rapidly as does the dependent tumor mass with antagonist or the sensitive tumor mass, without antagonist.

Resistance to a Guanine Analog, 8-Azaguanine

The triazolopyrimidine analog of guanine, 5-amino-7-hydroxy-1H-v-triazolo (d) pyrimidine, (8-Azaguanine), has been shown to be moderately inhibitory to certain bacteria (Roblin, R. O., *et al.*, 1945) and to *Tetrahymena* (Kidder, G. W., and Dewey, V. C., 1949) and a definite inhibitory action toward certain experimental tumors has been observed (Kidder, G. W., *et al.*, 1949) and confirmed (Gellhorn, A., *et al.*, 1950; Sugiura, K., *et al.*, 1950; Law, L. W., 1950). It is quite clear, however, that this metabolic inhibitor is entirely inactive against other tumors in mice and rats (Gellhorn, A., *et al.*, 1950; Sugiura, K., *et al.*, 1950). It has been suggested (Gellhorn, A., *et al.*, 1951) that the difference in response of tumor cells may be related to differences in 8-Azaguanine deaminase concentration. In certain acute lymphocytic leukemias of the mouse (Law, L. W., 1950), particularly the transplantable leukemia, L 1210, a definite, regular and reproducible inhibition of growth results from parenteral administration of the guanine analog at near-maximum tolerable doses. It was of interest, therefore, to determine whether resistance to this anti-leukemic compound, similar to resistance to anti-folics, could be developed.

Beginning with the 100th transfer of the sensitive line of L 1210 a standard dose of leukemic cells (8×10^5 cells) in Lockes' solution was inoculated subcutaneously in the right axillary region, into DBA strain mice, which subsequently received daily injections of 75 mg./kg. of 8-Azaguanine. (See text Fig. 2.) The guanine analog was dissolved in 0.01 N NaOH, and was injected subcutaneously on the left side, 0.05 cc. per gram body weight. Injections were begun 24 hours following transfer of leukemic cells and were continued until the next transfer. During the first 7 serial transfers, subcutaneous growths were obtained for further transfer at from 13 to 18 days. At the 8th transfer, however, palpable growths were obtained on the 4th post-inoculation day and at 9 days the mean weight of the lymphomatous mass was 573.9 mg. (Law, L. W., 1951). Subsequently, through the next 39 transfers, sizable tumor growths have been obtained in mice receiving a total dose of 600 mg./kg. of 8-Azaguanine, indicating the development of resistance. In contrast, identical transfer generations of the control subline, carried in untreated mice, remained sensitive to 8-Azaguanine, showing nearly complete inhibition of growth of lymphomatous tissue at 9 days.

Partial dependence for growth of these resistant leukemic cells on the guanine analog is shown in table 6. Significantly greater mean tumor weights were obtained in mice supplied with a total dose of 600 mg./kg. of 8-Azaguanine in contrast to mean weights of DBA strain mice growing the resistant cells but deprived of the analog. Differences in survival time of

TABLE 6

Effect of 3-Azaguanine on sensitive and 8-Azaguanine-dependent (8AG–D) lines of leukemia L 1210

Transfer line	Transfer generation	Number of mice	Dosage Daily	(mg./kg.) Total	Tumor wt. at 9 days (mg.)	Dependency ratio
Dependent	7–44 (107–144)	24(3)*	150	1,200	593.1	2.3
		183(25)	75	600	538.6±42.1	
		89(11)	None		240.4±21.8	
Sensitive	107–144	20(1)	150	1,200	0	
		53(5)	75	600	16.8± 2.2	
		54(6)	None		775.6±30.2	

*Numbers in parenthesis equal number of separate experiments.

mice growing the resistant line of leukemia L 1210, and supplied with or deprived of analog, also reflect a degree of dependence (table 7).

The morphology and biologic behavior of the 8-Azaguanine-dependent subline (8-AG-D) grown in DBA strain mice, rereiving 8-Azaguanine, resembles the sensitive control line grown in antagonist-free mice. At 9 days, in addition to growth of lymphomatous tissue subcutaneously, there results a florid leukemia with severe infiltration into lymph nodes, spleen and liver, leukocytosis with escape of lymphoblasts into the blood and a severe secondary anemia. The mean survival time of mice bearing the resistant and dependent cells and given 8-Azaguanine is strikingly similar to the control line. Table 7 gives the comparative blood picture of DBA mice growing the sensitive and dependent leukemic cells, the histologic pattern of infiltration and a comparison of the mean survival times of these groups.

This dependent subline has now been carried through 39 consecutive serial passages, since emergence of the trait, and has retained its characteristic response without any evidence of reversion. Optimal growth is obtained in mice receiving doses of 8-Azaguanine as high as 200 mg./kg. (1600 mg./kg. total dose). Inhibition in growth of these variant leukemic cells, similar to that occurring in the sensitive line, is produced by A-methopterin and TEM (Law, L. W., 1951).

Two closely related substances, known to be inhibitory for *L casei* (36), the dihydroxy-analog (5,7-dihydroxy-1-v-triazolo (d) primidine) and the diamino-analog (5,7-diamino-1-v-triazolo (d) pyrimidine)[4] have been tested in the sensitive and 8-Azaguanine-dependent lines. The diamino-analog was found to be ineffective in inhibiting the sensitive line or in providing growth in the dependent line. The dihydroxy-derivative, on the other hand, promoted more than 50% optimal growth in the 8-Azaguanine-dependent line but was found not to be inhibitory to the sensitive cells at concentrations as high as 200 mg./kg. $\times$ 8.

[4]These compounds were kindly provided by Dr. George Hitchings of the Wellcome Laboratories, Tuckahoe, New York.

TABLE 7

*Comparison in response of 8-Azaguanine-dependent and sensitive leukemic lines to 8-Azaguanine.**

	Line of leukemia	Dosage (mg/kg) Daily	Total	Survival Time, days	Hb	Wbc	Peripheral blood agran.	gran.	blasts	Organ infiltrations Lymph nodes	Spleen	Liver
1	8-Azaguanine dependent	75	675	9.8±.16†	10.3	127,150	(16%) 20,344	(43%) 54,675	(41%) 52,131	+++	+++	+++
2	sensitive	75	675	16.2	15	18,080	(53%) 9,582	(44.7%) 8,082	(2.3%) 416	—	—	—
	"	None		9.7	9.2	91,610	(30%) 27,483	(48.5%) 44,430	(22.5%) 19,697	+++	+++	+++

*Inoculations of a standard dose of leukemic cells (8×10^5) done intraperitoneally. All determinations are at 9 days post-inoculation. Blood and histologic studies represent means of 5 mice each and survival times were determined on 20 mice in each group.
†In contrast DBA mice growing 8-Azaguanine-dependent cells and deprived of analog had a mean survival time of 11.6±.31 days.

Partial reversal of the growth-promoting effects of 8-Azaguanine in the dependent line has been observed with fairly high doses of guanine HCl (375 mg./kg.).

It has been assumed by Kidder and co-workers (1949) that neoplasms, unlike normal tissues, require guanine for growth and that 8-Azaguanine acts as a competitive inhibitor of this changed metabolism of guanine by cancerous tissues. Results have been equivocal, however, relating to the question as to whether or not free guanine is metabolized by cancerous tissues in contradistinction to normal tissues (Brown, G. B., *et al.*, 1948; Brown, G. B., *et al.*, 1949; Graff, S., *et al.*, 1951). Mitchell *et al.* (1950) have shown that this anti-metabolite apparently enters into normal metabolic pathways. When mice were injected with 8-Azaguanine-2-C^{14}, or 4-C^{14}, radioactive 8-Azaguanine was isolated from both the RNA and DNA fractions of viscera and tumor tissue.

It is hoped that the variant leukemic cells described here, which are resistant to and partially dependent for growth on 8-Azaguanine will offer suitable material for studies on the rôle of guanine in cancer.

Origin of Resistance in Leukemic Cells to Folic Acid Antagonists

It appears that the changes to resistance and to dependence in the acute lymphocytic leukemias of the mouse occur rather commonly. Table 8 summarizes the transformations we have observed in 4 different transplantable leukemias and the change observed by Burchenal *et al.* in an Ak leukemia.

The pattern of resistance to a folic antagonist, A-methopterin has been determined using the same leukemia, L 1210, in DBA mice used previously. Beginning with the 110th transfer, of the sensitive (control) line, serial passage of leukemic cells was made in mice receiving graduated dosage levels of antagonist from 1.0 to 14.0 mg./kg. (total dose: 4.0-56.0 mg./kg.). It may be seen by reference to text figure 5 that increases in resistance have occurred in a discrete, stepwise fashion. Once resistance to low levels of antagonist has been attained, changes to resistance at higher dosage levels occur more rapidly. This particular line of leukemia, L 1210 (AM-R) (see text

TABLE 8

Transformations in leukemic cells of several transplantable lines.

Line of leukemia	Strain of mouse	Type of Transformation Resistant	Dependent	Antagonist used
Ak-4	Ak	+	—	A-methopterin
L 1210	DBA	+	+	A-methopterin
"	"	+	+	A-denopterin
"	"	+	?	A-ninopterin
L 1210	DBA	+	—	A-methopterin*
L 3054	C58	+	—	A-denopterin
HE 8186	A	+	?	A-methopterin
L 1210	DBA	+	+	8-Azaguanine

*Developed by consecutive serial transfers in mice receiving increasing doses of antagonist. Grows optimally at present in mice receiving 64 mg/kg A-methopterin. Other transformations developed by serial transfers of leukemic cells in mice receiving near M.T.D. of analogs.

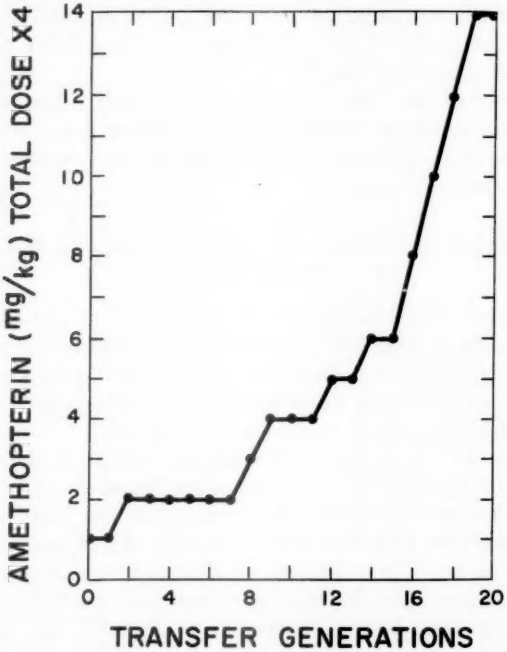

FIG. 5. Showing discrete, step-wise increments in the development of resistance in leukemia L 1210 to A-methopterin. Transfer of leukemic cells, 110th transfer of sensitive line, continued serially in DBA mice. This resistant subline (AM-R) now grows optimally at concentrations of analog as high as 56 mg./kg., total dose.

fig. 2 and table 8) remains resistant to high levels of A-methopterin (14.0 mg./kg. $\times$ 4), growing optimally at these levels. In contrast to the AM-D line, developed earlier from transfer 65 of the sensitive line, there has appeared no evidence in the line of dependence during 45 consecutive serial transfers. The pattern of resistance of leukemic cells thus resembles closely the pattern of penicillin resistance in bacteria (Demerec, M., 1948).

To explain the occurrence of the numerous variants among bacteria that grow in environments unfavorable to the parent strain (antibiotics, etc.) it may be assumed (1) that the unfavorable environment induces the change in some of the organisms, or (2) that the variant types arise as mutants during normal growth, the unfavorable environment being merely selective in their isolation. Luria and Delbrück (Luria, S. E. and Delbrück, M., 1943) utilized the 'fluctuation test' to distinguish these two possibilities. Using this experimental method on widely separated strains of bacteria, showing resistance to many different substances, numerous investigators have concluded that the variant type arose by spontaneous mutation (Newcombe, H. B., 1949). More direct evidence that spontaneous changes occur in the sensitivity of *E. coli* to phage (Newcome, H. B., 1949, to strptomycin (Newcombe, H. B., and Harvirko, C., 1949), and to chloromycetin (Cavalli, L. L., and Maccacaro, G. A., 1950) has been reported.

The fluctuation test was adapted to a study of the origin of resistance of leukemic cells to folic acid antagonist (Law, L. W., 1952). At the 105th serial transfer of the sensitive line of leukemia L 1210 in DBA strain mice, a small inoculum (150 cells) was transplanted subcutaneously into a DBA mouse. A large lymphomatous growth developed, and from this, fifteen independent transfer sublines were started. The independent sublines (1–15) were continued through six successive transfers in untreated mice. A standard dose of leukemic cells (8×10^5) in Locke's solution was used as inoculum in each case and all transfers of lymphomatous tissue were done at nine days.

At the seventh serial transfer of the fifteen independent sublines, standard doses of leukemic cells were then inoculated

subcutaneously into ten mice for each subline, and a total of four injections of A-methopterin (2.5 mg./kg. given every other day, beginning twenty-four hours post-inoculation). Subline 13 served as a control test. In this control series, ten groups of ten mice each were inoculated with the standard dose of leukemic cells and given A-methopterin. This subline, represents repeat tests of this population of leukemic cells. Thus, two different sets of tests were made: one in which leukemic cells were derived from fifteen independent sublines, and one in which leukemic cells were from the same (single) subline.

To determine whether resistant cells existed in the lymphomatous tissue used as the source, ten DBA mice were inoculated with a standard dose of leukemic cells from the 106th transfer that developed from the original inoculum of 150 cells. These mice were then given 2.5 mg./kg. of A-methopterin every other day for four days (10 mg./kg. total), the same dose used in all tests. No growths were obtained in these mice at nine days. It follows that any resistant cells found in the independent sublines arose during the subsequent seven serial transfers.

At nine days post-inoculation, animals were sacrificed and the lymphomatous tumor tissues weighed (see table 9). It may be seen that variation in the independent subline series, started from a common source, was highly significant. Two of the sublines contained no resistant forms. Variation in the single subline group was relatively small. The variance ratio between the independent and single subline series was 20, with $P<0.001$.

If resistant variants arose in this experiment by interaction of leukemic cell and antagonist, no significant difference among the various sublines of the independent and single groups would be expected. Approximately equal numbers of resistant forms should occur in each subline regardless of origin. On the other hand, if the origin of resistance was mutational, equal numbers of resistant forms should be obtained only among the samples tested in the single subline group, since these actually represent repeat tests. Assuming that mutations occur at random, there should be a highly significant fluctuation in the number of resistant forms in the independent subline group started from a common source.

TABLE 9

Variance analysis of the mean weights of lymphomatous tissue in independent and single sublines of leukemia L 1210

Independent sublines				Single subline			
Subline no.	Number of growths +	Number of growths —	Mean wt. (mg) lymphomatous tissue (9 days)	Subline no.	Number of growths +	Number of growths —	Mean wt. (mg) lymphomatous tissue (9 days)
1.	9	1	181.9	13a	6	4	22.0
2.	3	7	8.5	13b	5	5	67.7
3.	3	7	8.0	13c	6	4	51.0
4.	10	—	99.3	13d	8	2	54.5
5.	6	4	69.2	13e	6	4	58.0
6.	8	2	30.3	13f	6	4	36.2
7.	7	3	31.0	13g	6	3	41.4
8.	—	10	0.0	13h	8	2	47.4
9.	3	7	7.5	13i	8	2	27.0
10.	9	1	88.3	13j	8	2	42.4
11.	9	1	199.0				
12.	—	10	0.0				
14.	4	5	74.8				
15.	7	3	68.1				
	Mean	=	61.85		Mean	=	44.8
	Variance	=	4,144.84		Variance	=	195.9

It has also been found that tumors developed in uniform probability pattern in the test mice inoculated with leukemic cells of subline 13 (sample from a single subline): $X^2 = 5.29$; $P = 80$ per cent, while the proportions of leukemic and non-leukemic mice representing the independent sublines showed extreme variability: $X^2 = 61.85$; $P = 0.1$ per cent.

The results described: (1) extreme variation in the numbers of resistant leukemic cells among samples of independent sublines started from a common source; (2) increases in resistance in a step-wise fashion during serial transfer; and (3) stability of resistance in the absence of antagonist—would seem to favor the assumption that mutation and selection constitute the mechanism by which resistant-leukemic cells develop.

It is impossible to determine, with the present material, since these are somatic cells, whether the observed transformations are genetic. The two categories of mutants described, resistance and dependence, the former type of cell being able to grow optimally either in the presence or absence of antagonist, the latter growing optimally only in its presence, have maintained

their characteristics through many transfers, without reversion to sensitivity or change from one type to the other.

In *Escherichia coli*, strain K 12, a sexually fertile strain, it has been determined that the numerous changes to resistance and to dependence using streptomycin, appear to have arisen by change at a single gene locus (Newcombe, H. B., and Nyholm, M. H., 1950). Thus, these traits in bacteria behave as if controlled by allelic forms of the same gene locus.

Summary

Transformations to resistance and to dependence occur rather commonly among acute lymphocytic leukemias of the mouse. Folic acid antagonists and a guanine antagonist, 8-Azaguanine, have been used in the development of these variant forms.

Leukemic cells resistant to or dependent on folic analogs, are inhibited by other non-related anti-leukemic agents, but show a characteristic cross-resistance to all 4-amino substituted folic antagonists. Folic acid (PGA) and citrovorum factor do not influence the growth of the variant cells but both compounds inhibit the growth-promoting effects of A-methopterin in dependent leukemic cells.

These changes are shown to be stable, irreversible and heritable. No reversions to sensitivity or from one form to another have occurred. The pattern of resistance, to folic antagonist, resembles that developing in bacteria to penicillin. Discrete, step-wise increases in resistance occur in leukemic cells grown in mice receiving increasing doses of antagonist.

Experimental evidence favors the assumption that the variant cells arise by spontaneous mutation, which occurs constantly in the population of leukemic cells, the metabolic antagonist acting as a selective agent in the propagation of the variant forms.

The mechanism of resistance or dependence is not known. It is unlikely from available evidence, that production of adaptive enzymes or cellular mutations in the transformed cells allow for conversion of the analog to folic acid or citrovorum factor by the processes of deamination, demethylation, etc. It is more likely that the transformed cells used antagonist with-

out conversion, employing a different mechanism for the synthesis of nucleic acids than that suggested as occurring normally.

The rate of synthesis of nucleic acids has been studied in sensitive and dependent leukemic cells using C^{14} sodium formate. A-methopterin was found to approximately double the rate of DNA and RNA purine synthesis in the dependent tumor mass while profoundly inhibiting such synthesis in the sensitive tumor and in viscera.

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TUMOR RESISTANCE PHENOMENA*

I. Experimental Variables Altering Implantation and Growth of a Rat Fibrosarcoma

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Transplanted tumors of spontaneous or induced origin have been extensively used in research. This is undoubtedly the result of a general recognition that one of the most outstanding characteristics of tumors is their independence of growth, and that this phenomenon is common to all types of tumors, regardless of their origin. Propagation of tumor tissue outside the host of origin, whether *in vitro* or *in vivo*, has made it possible for experimentation to continue beyond clinical observations, with the result that many phases of the cancer problem now constitute rather specialized fields dealing with basic research on such subjects as chemotherapy and nutrition. Certain tumors have come to be regarded as standard in the armamentarium for these fields of experimental work, and each, when carefully used by experienced workers on a sufficiently large number of animals constitutes a reliable research tool.

Most workers who have used transplantable tumors have selected those which exhibit a high incidence of takes in a given host. Inasmuch as we were interested in the problem of resistance to implantation and growth, we intentionally used a tumor in which the incidence of successful implantation could be utilized as a criterion of effect. Through the use of this criterion, it is also possible to evaluate and standardize the technique itself. Thus, factors affecting viability of the transplant, such as peculiar environmental conditions, or metabolic changes within the tumor-bearing host or recipient host, could be set up for experimental testing. By carefully controlling experimental variables it is possible to separate resistant from susceptible hosts (Mefferd and Loefer, 1952a; Loefer and Mef-

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ferd, 1952a). We have used this method for both purposes and have segregated resistant and susceptible hosts for further biochemical and genetic study.

The immediate purpose of the experiments described here was to evaluate more fully the effect of certain variables in technique that conceivably might have a marked influence on implantation (Loefer, 1952a). While certain of these factors may have especial application in working with this tumor, yet the results clearly emphasize the necessity for rigid control of methods in work with all transplantable tumors.

As our studies on the phenomenon of resistance progressed, it became evident that a number of factors markedly affected tumor implantation. Some of these were inherent in the tumor, others within the tumor-bearing host, and still others resulted from purely environmental influences. Any one of these can determine success or failure of a tumor graft.

The tumor used, described as a fibrosarcoma by Dr. S. W. Bohls, has been propagated by subcutaneous transplantation in Sherman-strain rats, maintained on a Purina Laboratory Chow diet and kept in an air-conditioned room. The tumor is implanted ventro-laterally near either axilla or groin. Such factors as age of the host (Loefer and Gilles, 1951a), supplementary dietary factors (Loefer, 1951; Loefer and Gilles, 1951; Loefer and Mefferd, 1952) and age of the host bearing the tumor stock (Loefer, 1952), were previously shown to exert a significant effect on the number of takes.

This tumor usually attains a diameter of about one inch in two to three weeks and continues to enlarge slowly. Ulceration is rare, and up to 26 days there is seldom any evidence of necrosis at the center. We have not used tumors that were necrotic or ulcerated. Repeated streakings on a number of appropriate media have consistently failed to reveal either contaminating bacteria or fungi.

Results

Because vascularization often appears to be more extensive on the surface of a tumor than in its interior, it was of interest to determine whether tumor tissue fragments near the periphery differed in viability from those near the interior. In one experi-

ment, 254 rats, eight to fourteen weeks old, were segregated at random into two groups, evenly distributed with respect to males and females. Subcutaneous grafts were made with a trocar (1.6 mm. bore) from a 23-day stock tumor that had developed in a host that was twelve days old when grafted. Rats in the two groups were inoculated with peripheral or central material, respectively. Palpation 16 days later revealed data as indicated for series 1 in Table 1.

TABLE 1

Incidence of a transplanted rat fibrosarcoma. Center vs. periphery as a source of graft tissue. Graft tissue in each series was from the same stock tumor.

Series	Peripheral Stock			Center Stock	
	Age of rats	No. pos./total	% takes	No. pos./total	% takes
1	8–14 weeks	30/128	23.4	24/126	19.0
2	2– 3 weeks	53/89	59.6	53/84	63.1

A similar experiment (series 2, Table 1) with 173 rats two to three weeks old, although generally showing a considerably higher incidence of successful transplants, revealed that there was no significant difference in the viability of graft material from the two sources.

Zahl and Nowak (1949) reported that the edema resulting from traumatization of the implantation site was favorable to growth of a mouse sarcoma. In order to test this hypothesis, thirty-one male rats, five weeks old, were segregated at random into two respective groups. Test animals were given a local traumatization just prior to grafting by inserting a burr through the tube of the trocar and rotating it several times to effect laceration at the implant site. Duplicate grafts from a 21-day stock tumor were made on each animal, using a 3.4 mm. bore trocar. The results are shown in Table 2.

The results indicate that traumatization of the implant site had no significant effect on the incidence of takes. It is likewise evident that neither of the respective transplantation sites was better than the other.

In transferring tumor fragments to a large series of hosts there is often a considerable delay after removing the tumor, sectioning it, and inoculation of animals. In all our experiments

TABLE 2

Implantation of tumors into traumatized subcutaneous sites.

	Control	Test Group
Number of rats	15	16
Number of tumors developed	16	16
Number of transplant failures	14	16
Incidence of takes	16/30 = 53	16/32 = 50
Number of rats with two tumors	7	8
Number of rats with no tumors	6	8
Number of rats with one tumor	2 (1 on each side)	0

this factor has been equalized by alternating injections of the control and test animals. It was of interest to know whether certain environmental conditions might be detrimental to viability of tumor tissue during this period of unavoidable delay, and hence the following experiments were performed.

A 15-gram tumor was aseptically removed from a host, placed in a large sterile dry Petri dish and sectioned into 16 approximately equal pieces. Half of these were selected at random and transferred to a second wet Petri dish, *i.e.*, one in which the bottom was covered to a depth of 1/8 inch with physiological saline. All were then cut into smaller-sized units suitable for trocar loading. The two Petri dishes were kept at room temperature for thirty minutes, after which the tumor fragments were grafted into two respective groups of rats (58 in each group), one group receiving the "wet", the other the "dry" grafts. The elapsed time that the tumor fragments were subjected to these conditions averaged one hour. The number of tumors which implanted successfully in the two groups as determined by subsequent palpation, is shown in Table 3. There was no significant difference between the two types of treatment on viability. Successful grafts occurred as frequently among those inoculated during the last half hour as the first.

TABLE 3

Tumor implantation following exposure of stock to "wet" and "dry" environments.

Condition of Fragment	Total Rats	No. of Tumors	% Takes
"Wet"	58	46	79.3
"Dry"	58	43	74.3

In another experiment, 116 animals of similar age were segregated into two groups. Tumor fragments were held at 24°C. in one case and 37°C. in the other for an average period of one hour preceding implantation. Palpation of implants fifteen days later revealed results as shown in Table 4.

TABLE 4

Tumor takes following grafting with fragments kept at 24° and 37° C.

Temperature	Total Rats	Number of Tumors	% takes
24°	56	50	89.3
37°	60	39	65.0
(P = < .01)			

It was noted very early in work with this tumor that tissue fragments of a definite minimum size were essential for successful transplantation. It was impossible to transmit the tumor with non-cellular material such as obtained by homogenization with a Waring Blender. Grinding in a mortar in physiological saline in such a manner as to produce small clumps and numerous isolated cells was likewise unsuccessful, even though large amounts of the material were injected subcutaneously or intraperitoneally.

Within a certain size range, the percentage of takes tends to vary directly with the size of the implant, as the following series of experiments show. A total of 118 rats, segregated into three age groups, were given duplicate grafts subcutaneously, one large and the other small. A different stock tumor was used in each experiment. Comparative results, in which each animal served as its own control, were obtained by palpation 18–20 days later, and are shown in Table 5.

TABLE 5

Incidence of successful implants with 74 (left axilla) and 9.3 mg. (right axilla) grafts.

Expt.	Age of Rats	No. of Rats	No. tumors from 74 mg. graft	% with Tumors	No. tumors from 9.3 mg. graft	% with Tumors
1	3– 5	37	27	73	18	48.6
2	7– 9	40	22	55	10	25.0
3	9–11	41	18	43.9	11	26.8
Totals & Averages	.....	118	67	56.8	39	33.0

Table 6 presents data from experiments 1 and 3 (Table 5), which clearly demonstrates that the tumor graft size is critical. Animals resistant to the large (74 mg.) graft are, as expected, resistant to the small graft (9.3). These animals, which are highly susceptible, as evidenced by the successful implantation

TABLE 6

Distribution of takes in 78 rats from expts. 1 and 3. Number of rats are indicated in respective columns.

Tumors Developed	Exp. 1	Exp. 3	Totals	%
Two, one from each sized implant..	18	11	29	37
None	10	23	33	42
One, from large graft.....	9	7	16	21
One, from small graft.....	0	0	0	0

of the small tumor, likewise develop the large tumor in all cases. Moderately resistant animals developed only the large graft, while no small grafts could develop except in highly susceptible animals, *e.g.*, those developing both tumors.

Discussion

Our fibrosarcoma can be advantageously employed for the investigation of tumor resistance phenomena, provided that certain experimental variables are carefully controlled. These variables, as shown in this paper and in previous reports, influence the percentage of successful implants. Control of these has made it possible to use incidence of takes as a criterion for the selection of animals on the basis of resistance, as well as for analyzing the effect of experimental variables on host resistance to implantation.

The essential dichotomy of neoplasia, viz., tumorogenesis and tumor growth, calls for a method which can clearly differentiate between these two features. Since this is essentially impossible to accomplish with spontaneous or induced tumors, transplantable tumors, by means of which these features can be studied separately, are ideally suited for studies of this nature. A transplanted fragment of a tumor, such as that of our fibrosarcoma, consists of a mass of cells of measurable size, which after grafting, can be directly observed for subsequent changes. By varying the size of this implant, almost any percentage of takes

desired can be obtained. This is indicative of the fact that the defensive mechanisms of the host are able to neutralize a rather constant number of cells (Murphy, 1926). For all practical purposes, therefore, these ill-defined resistance factors may be eliminated from studies involving tumor growth (Mefferd and Loefer, 1952), by increasing the size of the fragment transplanted until all grafts implant. It has been shown for other tumors that the chances of establishing a transplantable tumor are proportional to the number of cells inoculated (Schrek, 1936; Shear, 1936; Reinhard, *et al.*, 1945; Zahl and Drasher, 1947). The increased "latent period" for a tumor-take observed after a small graft is made may simply be the result of the relatively few cells that were able to survive. The measured inoculum-size is probably an artifact in that the resistance forces of the host will destroy a certain rather definite number of cells in this inoculum. The true inoculum size, which determines the take and "latent period" (*i.e.*, that period required for the true inocula cells to attain palpable size), is actually the resultant of the measured inoculum size and the resistance forces of the host. It was established that grafts from any part of the tumor are equally potent. It may be assumed therefore, that the growth rate is constant throughout the tumor, a least during the early stages of tumor growth.

The trauma from trocar inoculation results in an accumulation of highly nutritious lymph, serum, etc., at the graft site (Zahl and Nowak, 1949). In view of this fact and that additional traumatization, as shown by our results, did not increase the incidence of implants, it is apparent that nutrition of the graft during the implantation stage is not the limiting factor for its establishment. Thus the defensive forces immediately mobilizable by the host are the determining factors in implantation. Although these forces are relatively constant in each individual (*i.e.*, additional traumatization elicits no additional defensive response), there is wide variation among individuals. If a tissue fragment large enough to insure 100% takes is used to completely overwhelm resistance forces, it is only reasonable to assume that implantation occurs within a relatively short period of time, perhaps within a few hours.

Not only is it impossible to determine the time of genesis of a spontaneous or induced tumor, but it is likewise impossible to determine the number of tumors that never achieve perceptible size. Hence, when the term incidence is employed in the conventional manner by workers using spontaneous or induced tumors, only those tumors are signified which possessed sufficient potency to have attained considerable growth after genesis. The number that appeared, but did not grow or that regressed, remains an enigma. To resolve the dichotomy of tumorogenesis and tumor growth, it is necessary therefore, to use either a group of animals bearing tumors of similar size, or to use a transplantable tumor in which the graft is of observable size.

In the latter case, it is also possible to study implantation as well as growth, since a graft must either disappear, remain unchanged in size, or grow. It must be recognized, however, that such implantation may be different from the genesis of induced or spontaneous tumors.

Not only does a transplantable tumor enable the investigation of the role of various agents upon growth and/or implantation, but since it conforms to the classical definition of a tumor in all senses (*i.e.*, an abnormal mass of tissue, not inflammatory and independent in character, arising without obvious cause from cells of pre-existent tissue, possessing no physiological function, and characteristically unrestrained in growth and structure), it may be used in many types of investigations on the tumor *per se*, or for the study of tumor-host relationships (Loefer and Mefferd, MS.c). It possesses the additional advantage that one is dealing with a similar initial population in the tumor graft, whereas this can never be said with assurance for induced or spontaneous tumors.

Summary

Studies designed to evaluate tumor resistance and factors influencing it must necessarily employ a transplantable tumor in an inbred strain of animals having resistant individuals. A rat fibrosarcoma, which has been carefully propagated so as to minimize cellular population problems and which possesses the desirable features of a constant growth rate for a period of at least three weeks yielding a firm tumor which has equal potency

for transplantation throughout the tumor, has proved to be ideal for resistance studies.

A maximal defensive response is elicited by trocar injection, and the ensuing edema of highly nutritious substances renders nutrition non-limiting. Therefore, implantation becomes directly dependent upon the level of the immediately mobilizable defensive forces the individual can muster, and, since all individuals differ in this respect, it is possible to segregate resistant animals. Implantation, being proportional to the fragment size employed, can be secured in any degree desired. It then becomes possible to segregate animals of almost any degree of resistance. This technique is dependent upon the procedures used, *e.g.*, viability is higher when the excised tumor is sectioned at 24°C. than at 37°C.

Only by use of a transplantable tumor can the dichotomy of tumorogenesis and subsequent tumor growth be resolved, since it is virtually impossible to determine the exact time of tumorogenesis, or even to detect it in the absence of a palpable tumor. By using a tumor fragment of measurable size, which overwhelms the defensive forces of the host and thus implants in a short time, it is possible to investigate tumor growth in a critical manner.

A more complete summary of tumor resistance phenomena and growth in relation to individual metabolic patterns is given elsewhere (Loefer and Mefferd, 1952b; Loefer and Mefferd, MS.d; Mefferd and Loefer, MS.b).

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TUMOR RESISTANCE PHENOMENA*

II. Intrinsic Resistance to Tumor Implantation

Roy B. Mefferd, Jr.,† and John B. Loefer

The incidence of takes following transplantation of a fibrosarcoma in Sherman-strain rats has been used in this laboratory for the investigation of host resistance (Loefer, 1951; Loefer and Mefferd, 1952; Loefer and Mefferd, 1952a; Mefferd and Loefer, MS.). Consistent variation in takes, depending upon experimental techniques used, has led us to believe that this procedure is a reliable indicator of susceptibility to implantation. The possibility that some factor, or factors, in the experimental technique might be influencing the results made it necessary to conclusively establish the existence of intrinsic host resistance, and to determine the reliability which can be placed upon the results obtained with single grafting. Likewise, it was desirable to determine whether the site of inoculation was a determining factor in tumor implantation.

In view of the fact that tumors can only be maintained by propagation from host to host, possible physiological degeneration or other variation within the tumor becomes important. Although it may be impossible to compare results between experiments conducted several years apart, it is desirable that the variation be of such a small order that comparisons can be made in a series of consecutive experiments, as well as from the results of a single experiment which is so large as to require several different tumor stocks. It is, therefore, essential to establish the reliability of such procedures.

Experimental

Fifty-six 14- to 16-week Sherman-strain rats, 31 males and 25 females, from our own colony, maintained as previously described (Loefer and Gilles, 1951), were used. Four stock tumors 16 days old were obtained from 4–5 week hosts. Trocar

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implantations were made from each tumor, using 9.3 mg. fragments, and those from each tumor were introduced into respective ventro-lateral subcutaneous sites near the right and left axillae and groins. The "dry" technique at room temperature, as previously described (Loefer, Mefferd, and Nettleton, 1952), was used. The time-delay factor was controlled by suitably reversing the order of inoculations. Four weeks later incidence of implantation was determined by palpation. The results obtained are shown in Table 1, and the distribution of implants from quadruple grafting is indicated in Table 2.

TABLE 1

Implantation of tumors in rats.

	Stock Tumor				
	A	B	C	D	Summary
Total tumors—male	13	14	11	9	47
Graft failures—male	18	17	20	22	77
Total tumors—female	12	11	8	10	41
Graft failures—female	13	14	17	15	59
Total takes	25	25	19	19	88
Total failures	31	31	37	37	136
% overall takes	44	44	34	34	39

Incidence for males = 37.9%; for females = 41%.

TABLE 2

Distribution of tumors in 56 rats which received quadruple grafts.

	Number of Tumors				
	None	One	Two	Three	Four
Males	17	1	1	4	8
Females	11	3	1	4	6
Total	28	4	2	8	14

Discussion

The overall incidence of implanted tumors was 39%, 41% in females and 37.9% in males, which difference is not significant as has been noted before (Loefer and Gilles, 1951). It will be noted that the respective incidence figures for tumors A, B, C, and D were 44, 44, 34, and 34% respectively. There was no significant difference between these extremes, indicating that (a) when stock tumors are grown in different animals

but under identical conditions, viability is not altered, and (b) relative position of implant site exercises no specific effect.

Of the total 56 animals, 28 (50.0%) were completely negative to all 4 implantations; 14 rats developed quadruple grafts, while less than 4 implanted in 14 animals. The latter group probably represents a certain uncontrollable experimental error, as will be shown later. This error is, however, relatively constant, throughout any experiment, and may be due to factors such as variation in manipulation, struggling of animals, and variation in sectioning the tumor and loading the trocar.

In Figure 1, a comparison between observed and expected frequencies of tumors in a carefully-controlled experiment is shown, based upon the following assumptions: 1. Individual rats are either totally susceptible or totally resistant to a graft of given size. 2. Any individual developing at least one tumor, but less than four, did so as a result of experimental error. 3. Rats developing no tumors may be omitted from the computations, since they are totally resistant. Such a result would be very unlikely to occur due to chance errors in technique. 4. The resulting population is a binomial distribution of the form,

$$(q + p)^4 = q^4 + 4q^3p + 6q^2q^2 + 4qp^3 + p^4,$$

where, if all animals in this population are considered to be susceptible, q^4 is the probability of zero takes, p^4 is the probability of quadruple takes, and the second, third, and fourth expressions are the probabilities of one, two or three takes, respectively. From this the expected class frequency is $N(q + p)^4$, where N is the number of susceptible rats in the population. When N is small, Nq is very small, and it does not appear to be unreasonable to assume that any animal showing four negatives is a highly resistant animal. The value of q after eliminating all quadruple negative animals is the ratio of the remaining graft failures to the total sample, or 4N in this case. Calculated in this manner, $q = 0.2140$, and therefore, $p = 0.7860$. The class frequencies, using these values, become 0.0, 0.9, 4.8, 11.6, 10.7 for 0, 1, 2, 3, and 4 takes, respectively, for the population in which at least one graft implanted. It may be noted from Table 2 that the observed frequencies were

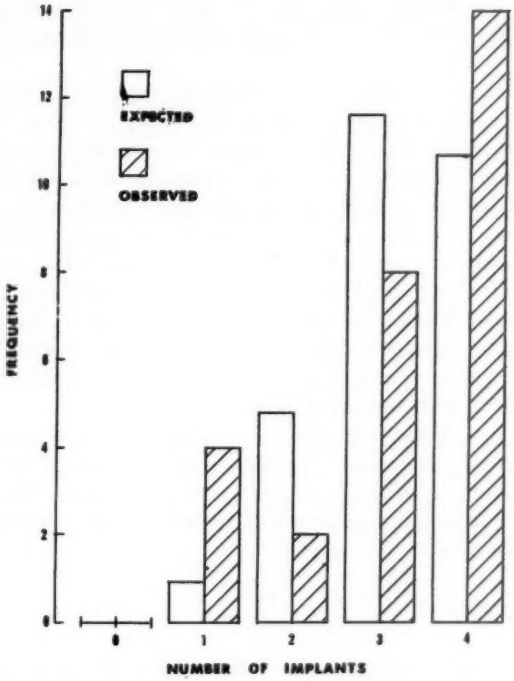

FIG. 1. Observed and expected distribution of implants in susceptible animals.

Note: Animals considered were those that received quadruple grafts which developed one or more tumors.

Calculation of expected values was based upon the assumption that distribution of grafts implanting would form a binomial population, *i.e.*, failure of fewer than 4 transplants to implant in a susceptible animal results from experimental error.

4, 2, 8, and 14 for 1, 2, 3, and 4 takes, respectively. The fit of the experimental results to the theoretical distribution yields a X^2 of 2.15. This value is not significant, and hence there is no reason to reject the assumption that the experimental data was obtained by sampling a binomial population, as previously defined. Thus, with the possible exception noted

below, an animal taking one or more tumors is intrinsically susceptible, and conversely, those animals taking no grafts were intrinsically resistant.

An important fact to be noted from Figure 1 is that the observed values are greater than the expected values in those animals taking one or four tumors. This lends credence to the idea that technical errors are involved. It is possible that several of those animals taking one tumor are actually resistant, but due to handling, chance receipt of a larger than normal-sized graft, etc., one tumor implanted. This would mean that the value for q used above was too large, and that p, therefore, was too small. It follows then, that $4qp^3$ in the binomial expression was too large, which in general is the way the expected values should be changed in order to obtain better agreement with the experimental results.

The fact that the incidence of any given tumor did not vary significantly from any other or from the average of the four indicates that the results in an experimental series from a single tumor inoculation are almost as reliable as those obtained from multiple grafts.

Summary

By using quadruple tumor inoculations in which four separate tumors were used, it has been demonstrated that neither the site of the graft, the sex of the animal, nor stock tumors from different donors of like age and bearing similar tumor implants, had any significant influence on implantation. The results from a single inoculation constitute a reliable means of segregating resistant animals, although, of course, it is not as accurate as the results from quadruple inoculations. It was demonstrated that there is an intrinsic resistance to tumor implantation.

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TUMOR RESISTANCE IN RELATION TO INDIVIDUAL METABOLIC PATTERNS*

I. Alteration of Host Susceptibility to Tumor Grafts by Nutritional Means

John B. Loefer and Roy B. Mefferd, Jr.†

Strains of rats in which a given transplantable tumor is so foreign that implantation incidence is low have not often been used for experimentation with that tumor. The exclusion of such hosts has precluded, *per se*, a valuable avenue of approach, since with such strains it is possible to investigate resistance phenomena, assuming that experimental variables are controlled.

We have experimentally investigated tumor resistance phenomena for a number of years, using a rat fibrosarcoma in which takes in Sherman-strain rats can be altered by variation in technique (Loefer, 1952a; Loefer, Mefferd, and Nettleton, 1952). In certain other strains, incidence is very high under identical conditions (Loefer and Mefferd, 1952a). Critical experiments have established the existence of an innate resistance to tumor implantation, and the results from a single graft are essentially similar to those obtained with multiple implants (Mefferd and Loefer, 1952).

When technical procedures are adequately controlled, resistance to implantation has been shown to be alterable by other factors. The age of the graft recipient, for example, was demonstrated to be influential, although sex, at least in relatively young rats, does not appear to play a significant role (Loefer and Gilles, 1951). Nutritional treatments such as supplementary injections of pyridoxine (Loefer, 1951) and thymic extract (Loefer and Gilles, 1951a) can markedly change resistance patterns. The viability of the tumor itself may be influenced by the age of the animal bearing it (Loefer, 1952b).

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Materials and Methods

Sixty rats, averaging 146 grams in weight, were segregated at random into three groups and placed in cages, five rats to a cage. Purina Laboratory Chow and water were supplied *ad libitum*. The twenty rats in the control group were each given two milliliters of physiological saline intraperitoneally daily for a period of thirty days. In a second group of twenty, each received, via the same route and on the same schedule, 10 milligrams of phenylalanine dissolved in a similar quantity of saline. This amount represented almost 100% above the normal daily intake for this amino acid. Rats of a third group each received 60 milligrams per day of β-2-thionylalanine in a similar manner.

After thirty days on these regimens, all rats were implanted subcutaneously with tumor fragments from a single stock tumor sixteen days old, taken from a male animal that had received the graft when 12 days old. With a trocar, the tumor fragments were placed ventrally near the right axilla. After this, each group was maintained on its respective regimen for two more weeks, when the tumors were palpated.

Results

Of nineteen surviving animals in the control group, fourteen (74%) had palpable tumors. In the phenylalanine-treated group, only 5/19 (26%) exhibited implants. There was an average weight gain of 5% in the control rats, and 14% in those which received supplementary phenylalanine. Within either group, however, there was no correlation evident between individual weight gain and susceptibility to the graft, *i.e.*, some rats which took tumors gained more, others less than the average for the group. The attempt to create a phenylalanine deficiency by injections of β-2-thionylalanine into Chow-fed rats was unsuccessful, since neither the rate of gain nor the incidence of tumor takes varied significantly from the values obtained for the control group.

Discussion

It is important to recognize that host-susceptibility to a tumor graft is a problem distinct from its subsequent growth and

development, the latter involving essentially a host-parasite relationship. Considerable work has been reported concerning the effect observed on the growth of tumor implants by an increase or reduction in quantity of nutritional factors (Stoerk and Emerson, 1949), and hormonal substances (Diller, Berk, and Blauch, 1948; Ladd, Goldstine, and Burns, 1950; Lewis and Aptekman, 1951; Loefer, 1952), but the manner in which such subtances influence the host with respect to its ability to exhibit resistance or tolerance to a graft remains a relatively unexplored field. Certain evidence indicates, however, that a low order of acquired resistance is established (Loefer and Mefferd, MS.b).

Earlier evidence showed that supplementary pyridoxine and thymic extract effected a decrease in host resistance, *i.e.*, enhanced susceptibility to tumor implantation. As shown in the present paper, however, incidence of implants was significantly lowered in rats that had received supplementary phenylalanine.

While the effect of supplementary phenylalanine on subsequent tumor growth was not determined, one might do so by using graft fragments sufficiently large to overcome host resistance, and measuring the tumors after a growth interval (Mefferd and Loefer, 1952a).

It is difficult, at present, to advance any hypothesis to account for these effects. A plausible explanation undoubtedly requires a better understanding of the individual metabolic patterns of these animals. Although one may suspect from the average weight gain figures that there is a correlation with host resistance, inspection of individual cases within each group indicates that there is none. The same was true for the earlier findings with respect to supplementary pyridoxine and injected thymic extract. Rather than assuming that these substances exert a direct effect, one must realize that such factors probably act indirectly in complex and diverse ways to alter individual physiological states, of which change in incidence is simply a reflection. This concept presents what we believe to be a valid working hypothesis (Loefer and Mefferd, 1952; *ibid.*, MS.c; Mefferd and Loefer, MS.a).

Previous workers (Ferger and DuVigneaud, 1949) have reported that rats fed β-2-thionylalanine (0.1-2.0% of diet) lost

weight, and that this weight loss could be reversed with phenylalanine. We gave intraperitoneal thionylalanine injections of sixty milligrams per rat per day, but were unable to demonstrate any weight loss even after 45 days of treatment. Even though this quantity constitutes only 0.4% of the normal daily food intake, it probably represents a greater effective level than the amount absorbed from even a 2% diet. Since the results of Ferger and DuVigneaud show that food intake dropped from 6–8 to 1.0–2.5 grams per day per rat when the analogue was mixed with the feed, the weight losses observed may be at least partially attributable to anorexia and starvation.

Summary

Supplementary administration of the essential amino acid phenylalanine significantly increased resistance to implantation of a rat fibrosarcoma. This enhancement could not be correlated with weight change. No effect could be demonstrated with β-2-thionylalanine, even though large quantities were administered over a 45-day period. In view of the fact that pyridoxine was shown to have a converse effect, it is clear that supplementation of this nature probably acts indirectly in complex and diverse ways to alter individual physiological states. Implantation in such hosts is only a reflection of this state.

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TUMOR SUSCEPTIBILITY IN RELATION TO INDIVIDUAL METABOLIC PATTERNS*

II. Influence of the Nutritional State of the Host on Tumor Growth

Roy B. Mefferd, Jr.,† and John B. Loefer

The role of nutrition in tumorogenesis has recently received considerable attention. Physicians since antiquity have believed that starvation constituted a treatment of cancer. Moreschi (1909) was the first to note that the growth of transplanted tumors could be inhibited by dietary changes. It was soon observed that overfeeding likewise stimulated tumor growth. Rous (1914) reported that recurrence of partially extirpated tumors was inhibited by a subnormal diet. Bischoq, *et al.* (1935), and Bischoff and Long (1938) demonstrated that caloric restriction, *per se*, inhibited tumor growth, and Tannenbaum (*cf.* 1947) reported that it inhibited the incidence of both spontaneous and induced tumors. The latter author correctly stressed the dichotomy of tumorogenesis and subsequent tumor growth, and also emphasized that any substance which affects the basic metabolism actually effects a change in the caloric ingestion. A considerable number of investigations since these discoveries were published have failed to alter the evidence that caloric restriction inhibits tumor growth.

The evidence for the influence of above-normal caloric intake is not quite so clear. White and Belkin (1945) found that animals upon a protein-deficient diet supported seventy-four per cent as much tumor tissue growth as did the control animals. The demonstration that the type of protein administered elicited different physiological responses (Guggenheim and Buechler, 1946 and 1948), and that in the rat, 4–8 weeks are required to deplete pre-existing protein reserves (Woolridge, *et al.*, 1950), necessitates re-examination of much of the protein-depletion work. Le Page, *et al.* (1952), report that fasting had no influ-

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ence upon tumor proteins, since the tumors grew even when the host was losing weight.

The role of the B vitamins in the growth of tumors has received a great deal of attention. Oftentimes contradictory effects have been noted upon both tumorogenesis and tumor growth, but much of the early work must be re-examined in view of the fact that the vitamins in pure form have been readily available for only a few years. It is also regrettable that much of the work failed to distinguish between genesis and growth of the tumor. Bischoff and Long (1939) found that specific supplementation (thiamin, riboflavin, or nicotinic acid) did not affect tumor growth, but that a supplementation with the vitamin B-complex markedly increased tumor growth. Bischoff and Long (1941) discovered that a B vitamin-free diet arrested tumor growth and the effect was reversible. Brabec (1935), Henkel ((1941), and Lewisohn, *et al.* (1941), found that dietary supplementation of riboflavin had no effect upon tumor growth. Inhibition of tumorogenesis by supplementary ribonflavin was noted by Kensler, *et al.* (1941). Laszlo and Leuchtenberger (1943) observed a decreased number of takes when riboflavin was injected with yeast extract. Russell (1945) reported that a depletion-repletion technique involving thiamin and riboflavin accelerated the induction of tumors derived from the nervous tissue, but not those from connective tissue. Schaefer, *et al.* (1950), reported that the addition of a low level riboflavin (1 mg./kg. diet) to a choline-deficient diet had little effect, but when as such as 20 mg./kg. was added, no tumors developed. Miner, *et al.* (1943), believed that riboflavin, *per se*, prevented the formation of tumors in animals on a deficient rice-bran diet which in itself stimulates carcinogenesis. Morris (*cf.* 1947) demonstrated a direct effect of riboflavin upon tumor growth and incidence. A deficiency decreased both, and vice versa. Stoerk and Emerson (1949), and Silverstone and Tannenbaum (1951), likewise found that a deficiency of riboflavin had no influence upon the incidence of spontaneous tumors.

Utilizing techniques developed in this laboratory (Loefer and Gilles, 1951; Loefer, Mefferd and Nettleton, 1952), which enable us to study separately the influence of nutrition upon tumor implantation and upon subsequent growth, we have studied such effects upon tumor growth.

Experimental

The fibrosarcoma described elsewhere (Loefer and Gilles, 1951; Loefer, Mefferd and Nettleton, 1952), was also used in this investigation. A preliminary experiment with 74 rats was conducted to establish the size of inoculum required to give 100% takes, the dose of isoriboflavin required to cause a loss in animal weight, and the amount of riboflavin required to allow a constant gain in weight.

Eighty-nine young male Wistar (Albino Farms) rats were segregated at random into five groups, placed in wire-bottomed cages in an air-conditioned room, and given free access to a synthetic diet which was free of A, D, and B-vitamins (General Biochemicals, Inc., vitamin B-complex test diet, consisting of sucrose 68%; Vitamin Test Casein [G.B.I.] 18%; vegetable oil [hydrogenated] 10%; and salt mixture, U.S.P., XIV 4%). This was supplemented with the following:

	mg/# feed
Choline	200.
Pantothenate	3.
Nicotinamide	3.
Pyridoxine	0.5
Thiamine Cl	0.3
Biotin	.03
PABA	5.0
Inositol	100.0

Vitamin A and D—2 drops/rat/week (Haliver Fish Oil, Parke, Davis, containing 60,000 U.S.P. units vitamin A, and 10,000 U.S.P. units vitamin D)

The groups were injected intraperitoneally according to the following protocol:

Group	Number of animals	Substances injected	Amount	Carrier
1	21	Riboflavin	0.02 mg/day	5% gum ghatti
2	22	Carrier only	2.0 ml	5% gum ghatti
3	15	Riboflavin	0.2 mg/day	5% gum ghatti
4	21	Atebrin*	2.0 mg/day	5% gum ghatti
5	10	Isoriboflavin†	2.0 mg/day	5% gum ghatti

*Atabrine Dihydrochloride (Quinacrine HCl), Winthrop Chemical Co., Inc., New York.

†Isoriboflavin furnished through the courtesy of Dr. C. C. Stock.

In 25 days, the animals on deficient diets (2, 4 and 5) were losing weight and evidenced typical deficiency symptoms. Those receiving atebrin were also losing hair. All were then inoculated with uniform-sized tumor fragments large enough to ensure 100% takes. All rats were weighed and sacrificed at 15 days when tumors were excised and weighed.

Results

In Figure 1, data on individuals of each series are plotted in progressive sequence with respect to the ratio of animal weight change to tumor weight change. The rats exhibit 3 types of behavior: (1) Those animals gaining, *e.g.*, groups 1 and 3, (2) those losing, *e.g.*, groups 2 and 5, and (3) the series 4 in which some animals gained while others lost weight. In Figure 2, the change in animal weight is plotted against individual

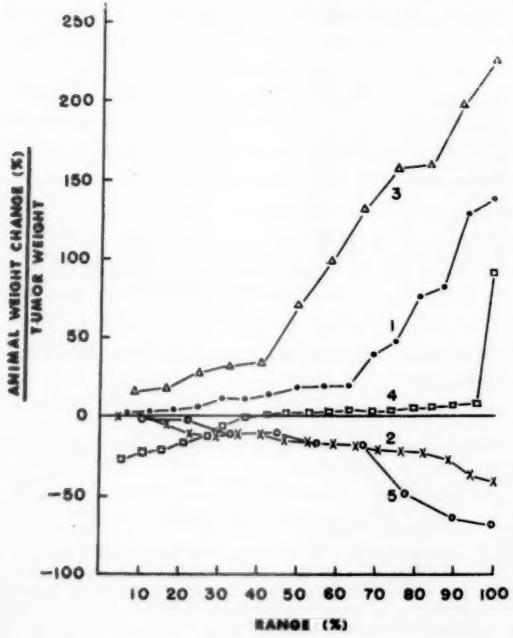

FIGURE 1

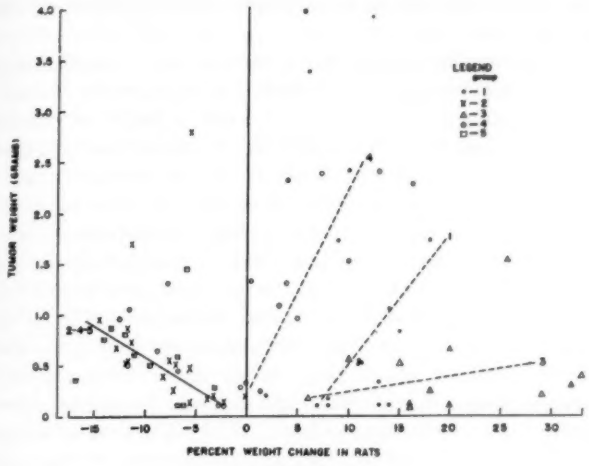

FIGURE 2

tumor weight. It may be observed that animals losing weight support tumors in inverse proportion to their weight loss. The animals gaining weight support tumors in direct proportion to the gain in weight. Atebrin, a substance which is partially competitive with riboflavin and partially a non-specific poison, inhibited the growth of only one half the animals in group 4. It is evident that whether animals in group 4 were gaining or losing, there is a trend toward large tumors in the rats undergoing the fastest rate of change in weight.

Discussion

The influence of a specific treatment upon tumor growth can be investigated only when tumorogenesis or tumor implantation is clearly excluded. The use of spontaneous or induced tumors for such work is *a priori* excluded, since it is impossible to determine the exact time of genesis or induction.

Likewise, when using a transplantable tumor, one of two alternatives must be observed, *i.e.*, the tumor must already have implanted at the beginning of the experiment, or a graft large enough to ensure 100% implantation must be used in order to

enable direct observation upon its course of development (Mefferd and Loefer, MS.).

Experiments involving the induction of a single vitamin deficiency are fraught with pitfalls for the unwary. It must be recognized that it is impossible to create a single vitamin deficiency in an organism. A multiplicity of deficiencies are immediately encountered. Stored supplies of the substance and its precursors and the products of its action are exhausted. Other related reactions become limiting. The animal often loses its appetite and a degree of inanition is established. In many respects the net effect amounts to a simple caloric restriction. Such a situation also finds varied expression in a complex organism such as a rat. The effects observed may arise from a direct or indirect action, *e.g.*, effect upon some part of the defensive mechanisms rather than upon the tumor directly. Another factor often overlooked involves the attempt to create inanition or caloric restriction. As the quantities of the various components are decreased, some substance falls below the minimum threshold requirement, thus creating an unintentional specific deficiency. The "low stave in the barrel" determines the adequacy of the diet.

Even among highly inbred animals, each is still a distinct individual with an independent genotype, and therefore has specific nutrient requirements, and hence, it is impossible to create an equalized deficiency in a group of animals. Each animal will be deficient, whether intentionally or unintentionally, to a different extent. Averaging results obtained from such experiments clearly does not present the true picture. Animals and data, in experiments of this nature, must be treated individually.

In the experiments reported here, a deficiency was created which was typical of the riboflavin-deficiency syndrome. Undoubtedly, a caloric restriction also resulted. Accompanying this multiplicity of symptoms there was a marked variation in the range of weight changes in the rats of the series. Tumors, however, developed in a striking manner as related to the actual extent of loss, *i.e.*, tumor size increased as body weight loss increased. This was the case whether the deficiency resulted from exclusion of riboflavin from the diet, or from injecting

large quantities of a competitive analog (isoriboflavin), or of a partially competitive analog of riboflavin (atebrin). The curves (Figure 2) are so similar that the actual causal agent must have a common basis. Animals given the normal daily requirement (Mannering, *et al.*, 1941) of riboflavin (0.02 mg./day/rat), were slightly deficient since the gains were not as great as when 0.2 mg./day/rat riboflavin was given. The trend toward the development of larger tumors as the rate of weight gain increases is distinct, but not as clear-cut as in the case of the deficient animals (*cf.* Figure 2). The same trend is evident among rats which gained, even though receiving atebrin.

We have attempted to interpret the complex relationships which obtain here on the basis of an interaction between mutally dependent and intercorrelated factors, and to reduce these to two components: resistance forces (R), and nutrition, including metabolic forces (N). If it be recognized that each of these is made up of at least several factors, then it is possible to consider the net result of each as a ratio, N/R. When these balance, *i.e.*, equal a value designated as unity (arbitrary values), the animals are not changing weight markedly and the tumors remain small. When the ratio becomes larger, however, the forces have become unbalanced.

In animals which are gaining weight at a high rate, the nutritive forces have increased, while the resistance forces have increased relatively less, since these have a tendency to remain constant (Murphy, 1926). Numerous studies have established that the resistance forces of the body, including both humoral and cellular mechanisms, are decreased sharply by deficient diets (Anderson and Fraser, 1934; Pinkerton and Bessey, 1939; Cottingham and Mills, 1943; Keys, *et al.*, 1945). It has been difficult, if not impossible however, to establish conclusively that improved nutrition enhances resistance, yet its dependence upon proteins in the diet (Cannon, 1942), and certain other indirect evidence leads one to believe that it does have an effect. Hence, it can be assumed that nutrition forces change at a more rapid rate than do resistance forces and thus the ratio tends to become larger when the animals gain or lose weight. In animals losing weight, nutritive forces decline rapidly, but resistance forces fall even more sharply. Thus the ratio again becomes large. This

concept is expressed schematically in Figure 3. While this explanation may not reveal the true picture, it constitutes a working hypothesis in that it simplifies to some extent the relationships which exist in such a complex situation.

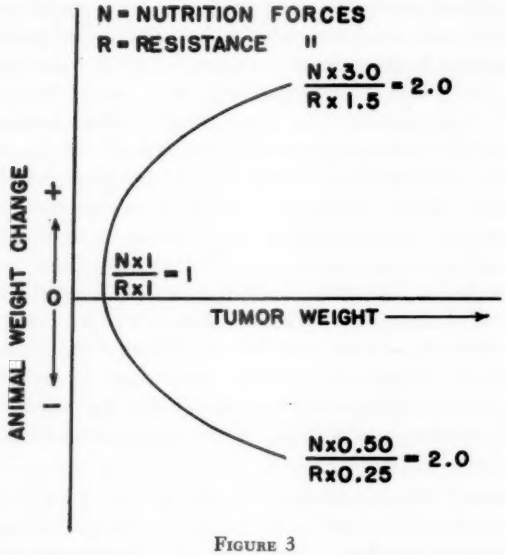

FIGURE 3

Other experiments (Loefer and Mefferd, 1952) have indicated that the amount of tumor growth supported varies markedly among different strain. This again indicates that individual metabolic variation manifested within a single strain is intensified between strains.

Summary

Experiments were designed to study the influence of nutrition upon growth of a rat fibrosarcoma. Respective groups of rats maintained on a riboflavin-free synthetic diet were administered supplements of 1) Normal riboflavin daily requirement, 2) Excess riboflavin, 3) Isoriboflavin and 4) Atebrin. It was demonstrated that there is an inverse relationship between the rate of animal weight loss and a direct relationship between the rate of

animal weight gain and the size of the tumor attained. A working hypothesis has been advanced in an effort to simplify the complex relationships involved between nutrition and resistance to tumor growth.

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TUBERCULOUS MENINGITIS*

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As with other forms of tuberculosis, interest in tuberculous meningitis has been stimulated tremendously by the application of streptomycin to its treatment, first reported in 1946 (13). It is our purpose to review the subject briefly placing emphasis on certain points which seem important from the large volume of recent literature and from our own experiences with four adult patients.

Case 1. 11721D, E. W. R., colored male, age 30. Admitted to John Sealy Hospital on June 15, 1951, after an illness of about one month. He had had intermittent periods of frontal headache and stiffness of the neck, with fever, nausea and vomiting, and during the week prior to admission, increasing somnolence.

Physical examination on admission showed a semistuporous, poorly nourished colored male. Temperature 100.4 rectally, stiff neck, hamstrung spasm and some engorgement of the retinal veins. Spinal fluid pressure 370 mm. of water. The fluid was slightly turbid with 90 WBC (41% neutrophiles, 59% lymphocytes); protein 89 mg. per 100 cc., glucose 28 mg., chlorides 326 mg. No organisms were found on smear or culture. Chest x-ray showed a finely scattered infiltration bilaterally.

In spite of streptomycin 2.0 Gm. daily, PAS 12 Gm. daily and penicillin 100,000 units every three hours, the patient continued to have fever, was increasingly stuporous and opisthotonic during the following two weeks. There were convulsions plus isolated clonic motions of his extremities. Beginning with the second week of therapy he was given 50 mg. streptomycin intrathecally each day.

Two weeks after admission he was started on pituitary adrenocorticotrophic hormone 20 mg. daily by slow intravenous drip, and within two days was asymptomatic except for residual neck stiffness. In spite of this the spinal fluid sugar and chlorides continued to fall to extremely low levels, the sugar averaging under 10 mg. and the chlorides 300 mg. On July 13th a hemolytic staphylococcus aureus was found in the spinal fluid and by the 15th spinal puncture was practically unsuccessful due to the thick pus present. Streptokinase and streptodornase[1] were injected intrathecally with marked and rapid clearing of the spinal fluid. Nevertheless, the patient progressed into coma and died on the 46th hospital day.

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[1]Streptokinase-Streptodornase supplied by Lederle Laboratories.

Post mortem examination confirmed the clinical diagnoses of pulmonary tuberculosis with acute hemotogenous dissemination; tuberculous meningitis; and pyogenic meningitis (staphylococcus).

Case 2. 9583H, C. W., colored female, age 57. This patient had complained of pain in her right chest for two months before her admission on August 8, 1951. Two weeks before admission her physician had removed an unknown amount of greenish fluid from the chest. On admission there was dullness and flatness over the right lower chest posteriorly. The patient was afebrile and did not appear to be acutely ill. X-ray examination at that time was suggestive of consolidation as well as fluid in the right lower chest. Thorcentes produced only 15 cc. of thick, opaque, greenish-yellow fluid from which a hemolytic staphylococcus albus was isolated. Bronchoscopy was negative. Skin test with O.T. 1:100 was positive. Two weeks after her admission the patient began to have daily elevations of temperature to 102. rectal, with a gradual increase to 103. and 104. just prior to her death. On September 5, 1951, one month after admission, she complained of severe headache, behaved irrationally and was found to have a stiff neck. Lumbar puncture was performed. A clear colorless fluid was obtained under an opening pressure of 240 mm. of water, contained 405 leukocytes with a differential of 83% polymorphonuclears and 17% lymphocytes. It contained 87 mg. protein and 398 mg. chlorides per 100 cc. Report of 100 mg. glucose was considered to be a laboratory error. Additional spinal fluid examinations on September 7th, 8th and 9th showed glucose as 28, 20 and 15, respectively. The protein, cell count and differential remained essentially the same. No organisms were found on smear of the fluid, although cultures later showed nonhemolytic staphylococcus albus coagulase negative. Although it was felt that this was a possible contaminant, the patient was treated with penicillin, gantrisin and later aureomycin without benefit. Patient expired on September 10, 1951, five days after the signs of meningitis developed. Post mortem examination revealed a diffuse tuberculous pneumonia and tuberculous meningitis.

Case 3. 2557H, O. P., colored female, age 27. Admitted to John Sealy Hospital on July 3, 1951. In January, 1951, she had had poliomyelitis with partial paralysis of both lower extremities. She returned for further care in this regard, as well as for her pregnancy, which terminated in a premature delivery on July 20, 1951. A right pleurisy with a small effusion developed on July 24, 1951. Two days later she developed a urinary tract infection which responded slowly to sulfonamide therapy. At this time she was transferred to the medical service. Her temperature, which had ranged as high as 103.8 prior to her transfer, continued to spike each day. Tuberculin skin tests were negative with O.T. 1:10.

On September 10, 1951, she complained of frontal headache. A lumbar puncture was performed. The opening pressure was 200 mm. water, the fluid contained 90 cells per cmm. with 69 polys and 21 lymphocytes, sugar

29 mg., chlorides 359 mg. and protein 187 mg. per 100 cc. Successive lumbar punctures were performed with essentially the same findings except for an increase in the number of white blood cells with a continuing predominance of polymorphonuclears. She received most of the antibiotics, with the exception of streptomycin, without influence on her clinical course or spinal fluid findings. At no time were pathogenic organisms discovered. Acid fast organisms were not present on smear or culture, although a pellicle formed on several occasions. On September 20, 1951, streptomycin 2 Gm. daily and sodium PAS 14 Gm. daily was started and by September 25, 1951, the patient was subjectively much improved, although the spinal fluid findings were essentially unchanged. Therapy was continued with gradual return of spinal fluid findings toward normal except for a persistently high protein. The last lumbar puncture was performed on January 18, 1952. The fluid was under decreased pressure, was deeply xanthochronic, and a pellicle formed almost immediately; chemistry is not available. Although all cultures were negative for acid fast bacilli, guinea pig inoculation was positive for tuberculosis.

It is planned to continue this patient's therapy for six months after her clinical recovery and then to plan further physiotherapy and orthopedic management of the lower extremity weakness.

X-ray examinations of the chest have not been entirely satisfactory due to the inability of this patient to stand, but scattered nodular infiltrates were noted through both lung fields in August and again on subsequent films.

Case 4. 1797H, R. F. C., Latin-American female, age 26. Admitted to Galveston State Psychopathic Hospital October 15, 1951, acutely ill, disoriented, confused, and almost stuporous. Temperature on admission was 102.4 rectally; there was rigidity of the neck, papilledema, right sided facial weakness, and hyperactive reflexes bilaterally. Spinal fluid showed ten cells per cmm. protein 100 mg., sugar 64 mg., chlorides (as Cl^-) 356 mg. per 100 cc. In the next two days the spinal fluid glucose was 48 and 40, respectively, and the chlorides 282 and 370, protein remained about the same but the cell count increased up to 77. Because the chest x-ray was suggestive of miliary tuberculosis the patient was transferred to the Isolation Hospital on October 18, 1951, for continuation of treatment. Dihydrostreptomycin 2 Gm. daily intramuscularly had been started on October 16th. This was continued and 50 mg. streptomycin given intrathecally each day after her transfer. She was also given sodium PAS 14 Gm. daily. Her temperature continued to spike to 103.° or 104.° each day and she varied between semistupor and coma. After one week of daily intrathecal streptomycin the dose was given every other day. Spinal fluid pressure, at first elevated, began to decline and it was necessary to tap at higher levels. With these taps, plus cisternal punctures, it was determined that there was a block at L-1 and probably a lesser block in the high cervical region. On November 10, 1951, the patient

was given 6800 units streptokinase and 2050 units streptodornase intrathecally. This was repeated on November 12th, 14th and 17th, and by November 19th it was possible to perform lumbar punctures at the level of L-3 or L-4 quite satisfactorily with crystal clear fluid and free dynamics. The administration of the streptokinase, streptodornase was spaced in order to prevent excessive rise in spinal fluid pressure. No effect on the patient's clinical course was noted other than that the block was definitely relieved.

Although the patient seemed to improve slightly for a time, she never really responded well to the streptomycin therapy and expired on November 28, 1951. On post mortem examination the clinical diagnoses of hematogenous tuberculosis (military type) and tuberculous meningitis were confirmed.

The use of streptomycin has modified the prognosis from uniformly fatal to possible recovery. Even so, results are far from ideal, with reported mortality in children ranging from 15% to 66% and from 50% to 90% in adults (7). Even with recovery, sequellae are frequent.

Lincoln (20) has emphasized the need for early diagnosis. Others (15, 21) report 50% survival in children when treatment was initiated before the tenth day of illness, but only 12% if started after the fifteenth day. Early symptoms include listlessness, apathy, sudden drowsiness and irritability. There may be fever, or a sudden rise in fever. Vomiting, apparently without cause, is frequent. Constipation, often emphasized as an early symptom, occurred infrequently in two reported series (16, 20). There may be remitting headaches which gradually become more intense. In some instances the disease may begin acutely with headache, delirium, stupor and convulsions (20% of children less than two years old in Lincoln's series).

During the early stages the neurological examination is normal. Later, the deep tendon reflexes are hyperactive with diminished abdominal reflexes. The Kernig and Bradzinski signs can usually be demonstrated; Babinski's sign is frequently present; there is often clonus at the ankle and knee. Occasionally there is evidence of cranial nerve involvement such as ptosis, nystagmus, strabismus, facial palsy. In the final stages there is coma, respiration is irregular, and the pupils become dilated and fixed.

In some instances the first suggestion for the diagnosis may come from the chest x-ray, the tuberculin reaction (positive in over 90% (16)), or the spinal fluid. Other than the presence of acid fast bacilli, the most important single finding in the spinal fluid is a low or falling sugar (below 38 mg. per 100 cc. in 96% (20)). The fluid is usually clear or slightly xanthochromic and comes out under increased pressure. The protein is elevated and a pellicle may form on standing. Chlorides are usualy decreased. The cells usually range from 10 to 350 per cmm., but may be over 1,000. Ordinarily lymphocytes predominate, but polymorphonuclear leugocytes may be in the majority, usually in association with rapidly progressive disease (20). This fact seems particularly noteworthy in view of cases 2 and 3 above.

Lincoln (20) also describes "serious tuberculous meningitis." This appears to be an acute exudate reaction in the meninges, without tuberculous infection. Clinically indistinguishable from tuberculous meningitis at the early stages, differentiation may be made from the spinal fluid findings. In this syndrome, there is an increase in pressure and slight pleocytosis but no change in the sugar or chlorides, and, early, the protein is normal.

Reaction to administration of PAS has caused confusion in the diagnosis in one case (3) where the patient developed fever, stiff neck and pleocytosis. Spinal fluid chemistry was normal. The syndrome subsided when PAS was discontinued.

In attempting to arrive at a diagnosis the previous occurrence of a pleural effusion of undetermined etiology should give rise to a strong suspicion of tuberculosis. In addition to the effusion preceding the meningitis in the cases described above, at least one report (18) calls attention to this as "the first evidence of reactivation and spread."

With regard to treatment there is general agreement that the dosage of streptomycin should be greater than that used for treatment of most other forms of tuberculosis. Recommendations range from total dosage of 1.5 Gm. (35) to 3.0 Gm. (7), intramuscularly, or 50 to 60 or even 100 mg. per kilogram of body weight per day. Bunn (7) has even suggested that a study be carried out using 6 to 8 Gm. daily in adults.

Toxic reactions following intrathecal administration of streptomycin have caused some concern regarding its use in this manner. These reactions include shock-like states, slow irregular respiration, increased vertigo, strabismus, projectile vomiting and temperature rise (20, 22). Rarely, there may be marked drowsiness and flaccid muscles, clearing within 24 hours. One death in respiratory failure and one case of coma with residual marked change in mental status have been reported (35). Even so, most authorities consider intrathecal administration essential (7, 24, 25, 26). The usual dosage is 50 mg. (12), although sometimes 100 mg. (7) is used, daily for seven to ten days, then every other day for two or three months. 2 mg. per kilogram is recommended for children weighing less than 25 kilograms (7).

At the present time only streptomycin can be used intrathecally, its dihydro derivative being too irritative and toxic for this mode of administration. For intramuscular use the Clinical Subcommittee on Medical Research and Therapy of the American Trudeau Society states that there is no choice between streptomycin and dihydrostreptomycin (12); the Veterans Administration Committee on Streptomycin prefers streptomycin to the dihydro form (22).

Various adjuvants have been employed in attempting to improve the mortality rate and reduce the incidence of sequellae. The use of PAS is common. Lincoln (20) long ago reported on results with streptomycin and Promizole; others have used other sulfones (11, 25, 31).

Cairnes and his co-workers (8) have reported surprising results in a few cases with intrathecal tuberculin as an adjuvant. Study in a larger series is under way.

Cathie (10, 11) has used streptokinase intrathecally with streptomycin, with much better results than with the use of streptomycin alone. His series is very impressive. Another British author (23), however, states that streptokinase appeared to be of no special benefit. Cathie (11) and others (35) have resorted to intraventricular administration of streptomycin when more commonly used routes were blocked.

Kinsell (19) has briefly mentioned the use of ACTH in combating severe toxicity from tuberculous meningitis. He emphasizes the need for concurrent intensive chemotherapy. Most of us are already aware of the effect of ACTH and cortisone on infections. At least one case of tuberculous meningitis has been reported following the use of cortisone in treatment of another disease (14).

The combined use of streptomycin and potassium iodide has been reported by Roque and Cleve (29). Their results were certainly better than with streptomycin alone but too few cases were treated to warrant any conclusions.

Only one report of treatment with neomycin was found (17); the patient died. No reports on use of viomycin were noted.

Most authorities stress the need for long follow-up in apparent "cures," because of the possibility of late recurrences. Bunn (5, 6) reports five deaths from relapses in 24 "recovered" cases in eight months after cessation of therapy. One more relapsed and died during a two and a half year follow-up. Cainan (9) reports similar results in long term follow-up study.

It is well known that "cure" frequently does not represent complete recovery. In addition to sequellae from drug toxicity such as vertigo or deafness, the usually mentioned sequellae include minimal hemiplegia, retarded mental development, intellectual defects and spastic paralysis (6, 9, 32). There have been a few reports of disturbed endocrine function including four instances of dystrophia adisposogenitalis (4, 32). In explanation of these sequellae, pathologists have reported new findings in addition to the previously recognized obstructive hydrocephalus, as a result of the fibrin deposits around the brain stem (6, 34). There is an acute arteritis in the involved area, rapidly transformed under streptomycin therapy to a proliferative endarterial reaction with severe stenosis or occlusion of the arteries (1, 28, 36). This results in multiple ischemic softenings, consistently found in the basal ganglia and internal capsule. As noted above, streptokinase and tuberculin, among other agents, have been employed with streptomycin in an effort to reduce the instance and severity of these changes.

Summary

Streptomycin has markedly changed the course and prognosis of tuberculous meningitis.

Best results are obtained with early diagnosis and intensive treatment over a long period of time.

Various adjuvants to streptomycin have been employed in an effort to reduce the incidence of sequellae, as well as to improve the recovery rate.

Long-time follow-up is necessary because of the frequent late relapses.

Addendum

In an effort to relieve the spinal block in case 3 above, streptokinase 5,000 units and streptodornase 1,700 units, in 0.7 cc. solution, were injected intraspinally every other day for five doses, without effect except for severe lumbar pain following the first injection. In view of the results obtained in other cases it is postulated that the lack of effect may have been due to the duration of the block.

On March 13, 1952, a cisternal puncture revealed crystal clear fluid under a pressure of 120 mm. water with 2 WBC per cmm., protein 10 mg., glucose 56 mg. and chlorides 406 mg. per 100 cc. No acid fast bacilli were seen on smear.

It is planned to continue this patient's antimicrobial therapy for another six months.

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FORMATION OF HISTAMINE FROM CARNOSINE AND HISTIDINE BY *ESCHERICHIA COLI*•

JOE B. NASH†

Hefter (1925) studied the decomposition of carnosine and histidine in the presence of a variety of micro-organisms in order to define the importance of these imidazole derivatives as precursors of human autointoxication products. All spp. of the enteric group investigated were found to decompose histidine. Whereas inoculations of intestinal flora sometimes caused breakdown of carnosine, all pure cultures investigated, with the exception of *Bacillus pyocyaneus*, were without effect on the dipeptide. These spp. included: *Eberthella typhosa*, *Salmonella paratyphi*, A and B, *Shigella paradysenteriae*, *S. enteritidus*, *Escherichia coli*, *B. subtilis*, *B. mesentericus*, and *Proteus vulgaris*. The end-products from the action of *B. pyocyaneus* were NH_3, acetic acid, and butyric acid, all relatively non-toxic compounds. Hefter concluded that carnosine is broken down completely or not at all, contrasting with the usual production of histamine from histidine. These results have been interpreted by du Vigneaud and Behrens (1939) as indicating the inability of most micro-organisms to split carnosine into its component amino acids.

In attempting to find clues as to the method of biological breakdown of carnosine, Parschin (1933) used this peptide as a sole source of nitrogen in cultures of yeast and *Oidium lactis*. A small amount of carnosine was recovered unchanged from the culture media, but no intermediates in the decomposition were isolated. Histidine had been shown by Ehrlich (1911) to be converted to imidazole ethyl alcohol, under similar conditions, by brewer's yeast, and imidazole propionic acid was isolated by Kiyokawa (1933) from cultures of *Oidium lactis*; he

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found that β-alanine is transformed into hydracrylic acid by this organism.

Experimental

In light of recent advances of establishing an adaptive decarboxylase activity in certain micro-organisms, the effect of washed suspensions of *E. coli* on carnosine was estimated.

The *l*-carnosine and carbobenzoxy-*l*-carnosine used in these experiments were synthesized in this laboratory by the method of Sifferd and du Vigneaud (1935). Histidine HCl · H_2O was obtained from Eastman Kodak Company, Rochester 4, New York, and purified, or isolated from beef blood hydrolysates.

The Production of Histamine from Carnosine by Washed Suspensions of E. Coli.—The ability of washed suspensions of *E. coli* to produce histamine from carnosine was investigated employing the methods of Gale (1940). Histidine was used as a control to ascertain the relative activity upon these two substrates. Koessler, Hanke, and Sheppard (1928) postulated that certain organisms produced histamine adaptively to buffer acidic metabolic by-products produced by these organisms in certain media. Gale (1940) elaborated upon optimal conditions for the decarboxylation of several amino acids by different bacterial species.

A broth containing 1% Difco tryptone, 2% glucose, and 0.3% beef extract was buffered with 0.05 *M* Na_2HPO_4-NaH_2PO_4 at pH 5.5. The broth was inoculated with a 24-hour broth culture of *E. coli* and incubated at 27° C. for 16 hours. The culture was shaken and eight 10 ml. portions pipetted into heavy 15 ml. conical centrifuge tubes and centrifuged at 3,500 r.p.m. for 10 minutes. The supernatant was decanted, 3 ml. of water added, the tube stoppered and shaken, the stopper removed, the tube recentrifuged as before and the wash water decanted. The organisms were suspended in the amounts of water shown in Table I. Carnosine and histidine were added. 1.0 ml. 0.15 *M* K acid phthalate buffer solution, pH 4.0, was added to tubes Nos. 1–6. N_2 was bubbled through the solution in each tube for one minute, the tubes quickly stoppered and shaken.

TABLE 1

The Hydrolysis and Decarboxylation of Carnosine by Suspensions of E. Coli.

Tube	Substrate	Buffer ml.	Water ml.	Histamine produced γ
1.	Histidine HCl · H_2O 4.6 mg./0.5 ml. H_2O	Potassium Acid Phthalate 1.0	1.5	47.5
2.	Histidine HCl · H_2O 4.6 mg./0.5 ml. H_2O	Potassium Acid Phthalate 1.0	1.5	52.4
3.	Control	Potassium Acid Phthalate 1.0	2.0	0.15
4.	Control	Potassium Acid Phthalate 1.0	2.0	0.15
5.	Carnosine, 5 mg./0.5 ml. H_2O	Potassium Acid Phthalate 1.0	1.5	22.5
6.	Carnosine, 5 mg./0.5 ml. H_2O	Potassium Acid Phthalate 1.0	1.5	22.5
7.	Carnosine, 5 mg./0.5 ml. H_2O	Phosphate 0.1 ml. for 30 min.	1.5	9.75
8.	Carnosine, 5 mg./0.5 ml. H_2O	Phosphate 0.1 ml. for 30 min.	1.5	6.75

Tubes 7 and 8 were incubated after buffering at pH 8.5 with 0.1 ml. of 0.05 *M* Na_2HPO_4 for 30 minutes at 27° C. under N_2. The pH of these tubes was adjusted to 4.0 with 0.7 ml. 0.15 *M* K acid phthalate buffer solution and 0.4 *M* HCl; the volume was adjusted to 3.0 ml. with distilled water. N_2 was bubbled through these tubes as in the case of tubes Nos. 1–6. All tubes were incubated for a total of 2 hours.

The suspension was centrifuged at the end of incubation; the supernatant liquid was poured into small vials and neutralized with 0.8 *N* NaOH using phenolsulfonephthalein as an indicator. The samples were assayed for histamine by the method of Code (1937).

E. coli is capable of hydrolyzing carnosine and decarboxylating the histidine liberated under the conditions of this experiment. Previous workers were not aware, at the time of their studies, of the critical pH range that must be maintained to accomplished this decarboxylation. Gale (1940) showed that little activity was observed below pH 3.5 or above pH 5.0. As shown by Table I, the conversion in the case of carnosine was not as complete as seen with histidine. On an equimolar basis there is more histamine produced from histidine.

The possibility that the bacterial peptidase which hydrolyzed carnosine is similar to the carnosinase of higher animals described by Hanson and Smith (1949) was investigated. Tubes 7 and 8 were buffered at pH 8.5 to estimate the hydrolytic cleavage of carnosine at this pH. If more optimum conditions were created, they were overcompensated by a decrease in the adaptive decarboxylase or increase of diamine oxidase activity and are not apparent in the amount of histamine produced in this experiment.

The diminished production of histamine in tubes 7 and 8 might also have been due to an inactivation of the metalloprotein complex that constitutes the active enzyme. This experiment was repeated using barbital as the buffer, which has been shown by Hanson and Smith (1949) to be without inhibitory effects.

The Effect of Barbital Buffer, Manganese, and Carbobenzoxy-l-carnosine on the Hydrolytic Activity of E. Coli Suspensions.—As barbital buffer and Mn have been shown to

afford optimum conditions for the activity of carnosinase, this experiment was designed to ascertain the effects of these compounds upon the dipeptidase that is known to be present in *E. coli*.

Carbobenzoxy-*l*-carnosine was studied as a possible antagonist to the hydrolytic splitting of carnosine. Hanson and Smith (1949) showed that the carnosinase of mammalian tissues is incapable of splitting this compound.

E. coli cultures were grown, centrifuged, and washed as in the previous experiment. The substrates added were dissolved in 0.5 ml. of distilled water. The organisms were suspended in 0.03 *M* K acid phthalate buffer, pH 4.0, and 0.03 *M* barbital buffer, pH 4.0; 1.0 ml. of buffer solution was added to each tube as indicated in Table II. 1.5 ml. 0.01 *M* $MnCl_2$ were added to tubes 5 and 6. Water was added to all tubes to make a final volume of 3 ml. The tubes were shaken after the addition of the substrate and incubated at 27° C. for 2 hours under N_2. The tubes were centrifuged, the supernatant decanted, neutralized and assayed as described above.

The use of barbital buffer instead of phosphate buffer apparently does not increase the activity of the dipeptidase of *E. coli*. As compared with the preceding experiment, there is a notable decrease in the amount of histamine produced.

Mn completely inhibited the formation of histamine under the conditions established.

E. coli, unlike the mammalian spp. investigated by Hanson and Smith (1949), has the capacity to hydrolyze carbobenzoxy-*l*-carnosine. The hydrolysis of this compound is apparently inhibited by carnosine.

The Effect of Histidine Decarboxylase Inhibitors on Histamine Formation by Suspensions of E. Coli.—The effects of varying concentrations of betaine hydrazide HCl and Na suramin were studied on suspensions of *E. coli* with added histidine. The betaine hydrazide HCl used in this study was prepared by the method of Girard and Sandulesco (1936), with the assistance of Miss J. K. Howard. A yield of 87% was obtained with a m. p. of 182° C. The compound was very hygroscopic and was kept in a vacuum dessicator. Small samples were crystallized from absolute alcohol before use.

TABLE 2

The Effect of Barbital Buffer and Manganese on the Hydrolytic Activity of E. Coli Suspensions on Carnosine

Tube	Substrate in H_2O	Buffer, 0.03 *M* ml.	H_2O ml.	$MnCl_2$ 0.01 *M* ml.	Histamine produced γ
1.	Histidine HCl · H_2O 4.6 mg./0.5 ml. H_2O	KH phthalate 1.0	1.5		40.4
2.	Histidine HCl · H_2O 4.6 mg./0.5 ml. H_2O	KH pthhalate 1.0	1.5		40.0
3.	Control	KH phthalate 1.0	2.0		0.3
4.	Control	KH phthalate 1.0	2.0		0.3
5.	Carnosine 5 mg./0.5 ml.	Barbital 1.0		1.5	0
6.	Carnosine 5 mg./0.5 ml.	Barbital 1.0		1.5	0
7.	Carnosine 5 mg./0.5 ml.	Barbital 1.0	1.5		4.0
8.	Carnosine 5 mg./0.5 ml.	Barbital 1.0	1.5		3.5
9.	Carbobenzoxy-*l*-carnosine 5 mg./0.5 ml.	KH phthalate 1.0	1.5		20.0
10.	Carnosine 5 mg./0.5 ml. carbobenzoxy-*l*-carnosine 5 mg./0.5 ml.	KH phthalate 1.0	1.0		6.0

E. coli was cultured on the same media as above except that it was buffered at pH 5.0 with 0.05 *M* phosphate buffer. A 48-hour culture was used in this experiment. Eight 10 ml. portions of the culture were pipetted into heavy 15 ml. conical centrifuge tubes and centrifuged at 3,500 r.p.m. for 10 minutes. The supernatant was poured off, and another 10 ml. portion of the culture was added and recentrifuged. An equivalent of 20 ml. of the culture was represented in each tube. The organisms were washed with 3 ml. of water, centrifuged and the wash water decanted. 10 mg. of histidine $HCl \cdot H_2O$, dissolved in 0.5 ml. water, were pipetted into each tube subsequent to the addition of betaine hydrazide, suramin, buffer and water. The total volume in each tube was 3.0 ml. The tubes were shaken and incubated under N_2 for 2 hours. After incubation, the cultures were centrifuged; the supernatant was poured off, neutralized, and assayed.

As shown in Table III histidine decarboxylase is inhibited by 10^{-3} *M* betaine hydrazide HCl.

TABLE 3

The Effect of Histidine Decarboxylase Inhibitors upon Suspensions of E. Coli with Histidine as the Substrate

Tube No.	Inhibitor	Histamine produced γ
1.	Betaine Hydrazide HCl 0.1 *M*	8.1
2.	Betaine Hydrazide HCl 0.01 *M*	8.0
3.	Betaine Hydrazide HCl 0.001 *M*	39.0
4.	Suramin Na 0.01 *M*	150.0
5.	Suramin Na 0.001 *M*	200.0
6.	Suramin Na 0.0001 *M*	200.0
7.	Control	165.0
8.	Control	155.0

10^{-3} — 10^{-4} *M* suramin solutions decreased diamine oxidase activity, as there was more histamine in these samples than in the controls containing no inhibitor. A dual inhibition of histidine decarboxylase and diamine oxidase had been described previously by Werle (1940) for betaine hydrazide and suramin in isolated mammalian tissue studies, but apparently suramin does not modify the histidine decarboxylase activity of *E. coli*.

Summary

The conversion of carnosine to histamine by *E. coli* in media buffered to pH 4 is quantified and compared with the conversion of equimolecular quantities of histidine similarly treated. Betaine hydrazide and suramin Na were employed; both are effective as histidine decarboxylase inhibitors *in vitro* on mammalian tissues, but suramin Na was found to be without activity on this bacterial decarboxylase. In *E. coli*, the hydrolytic enzyme capable of splitting carnosine is also different from the comparable mammalian enzyme, which is the carnosinase described by Hanson and Smith (1949).

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PROCEEDINGS OF THE TEXAS BRANCH OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

Spring Meeting

The University of Texas Medical Branch
Galveston, Texas, May 16–17, 1952

TITLES

Some Laboratory Findings on an Outbreak of Ornithosis. Thelma D. Sullivan, Malcolm Denley, and J. V. Irons, Texas State Health Department, Austin, Texas.

Production of Immune Serum Against Erythrocytes of Sickle Cell Anemia. Rose Schneider, University of Texas Medical Branch, Galveston, Texas.

The Bactericidal Action of Human Sera from Acutely Ill Patients, and Its Relation to the Antiproteolytic Power of the Sera. Runell C. McRoberts and Kenneth L. Burdon, Baylor College of Medicine and Veterans Administration Hospital, Houston, Texas.

Congenital Anomalies in Chick Embryos Used as Controls in Virus Research. L. C. Murphy, 4th Army Area Medical Laboratory, Fort Sam Houston, Texas.

The Metabolic Role of Lipoic Acid (Pyruvic Oxidation Factor). Gerald Seaman, University of Texas Medical Branch, Galveston, Texas.

The Increasing Resistance of *S. aureus* to the Major Antibiotics. Charles D. Dukes, A. M. Dukes, and C. M. Willard, University of Texas Medical Branch, Galveston, Texas.

Anti-Rickettsial Activity of a New Synthetic Antibiotic. Ludwik Anigstein and Dorothy M. Whitney, University of Texas Medical Branch, Galveston, Texas.

Effect of Temperature of Storage of Virulence of Dehydrated *Hemophilis pertussis* Cultures. R. H. Hanna, Louis Gerhardt, and Caroline Stearns, Texas State Department of Health, Austin, Texas.

Preliminary Report on the Clinical Use of a New Pertussis Antigen. Harriet M. Felton, University of Texas Medical Branch, Galveston, Texas.

The Management of Bacterial Infections of the Skin. Clarence Livingood, C. E. Head, R. M. Sautter, and J. Fred Mullins, University of Texas Medical Branch, Galveston, Texas.

Studies on Sporulation in *Clostridium nigrificans*. Allen I. Laskin and O. B. Williams, University of Texas, Austin, Texas.

Adaptation of Voges-Proskauer Reaction to Assay of Streptomycin, Jean Szafir, University of Houston, Houston, Texas.

Bacteriostatic and Bactericidal Effect of "DETT." Robert A. Brown and Frank B. Engley, Jr., University of Texas Medical Branch, Galveston, Texas.

Activation of Serum Protease in Man and Animals Following Exposure to X-Ray. Kenneth L. Burdon, Rufus K. Guthrie, and Shirley R. Faseler, Baylor College of Medicine, Houston, Texas.

The Influence of Incubation Temperature and Time Upon the Plate Count of Raw and Pasteurized Milk. I. I. Peters, A. and M. College of Texas, College Station, Texas.

Preliminary Studies of the Effect of Aureomycin Hydrochloride on Fixed Rabies Virus. D. J. Hahn, M. K. Dulaney, and E. B. M. Cook, Texas State Department of Health, Austin, Texas.

Studies on Production of Chick Chorio-Allantoic Membrane Culture Smallpox Vaccine. Caroline Stearns, Richard T. Wyatt, and E. B. M. Cook, Texas State Department of Health, Austin, Texas.

I. M. Lewis, Lecturer
Dr. Esmond E. Snell
The University of Texas
Austin, Texas

Lecture Topic: "Amino Acids in Bacterial Metabolism"

Lunch-Time Seminar
on "Biological Warfare." Moderator—O. B. Williams

Routes of Infection—Robert Pike
Role of Insects—Don W. Micks
Plant Diseases—C. C. Doaks
Animal Diseases—Morris Pollard
Medical Intelligence—J. V. Irons
Decontamination Procedures—F. B. Engley

Abstracts

The Fixation of Anti-organ Antibodies *in vivo* as Determined by Radioactive Iodine 131. Ludwik Anigstein, K. P. McConnell, P. Pappas and Dorothy Whitney. The University of Texas Medical Branch, Galveston.

Antiorgan rabbit immune sera against rat organs (liver, spleen, kidney, pancreas, lungs) were fractionated and the globulin fraction labelled with radioactive I^{131} for the purpose of tracing the distribution of antibodies in the tissues of the injected rats. The radioactivity of the organs was evaluated by Geiger-Muller counter on the basis of the ratio of counts per minute per gram (cpm/g) of the homologous rat tissue to cpm/g of heterologous tissue. In rats injected with tagged antiliver globulins, the radioactivity of the liver was found exceeding that

of any other organ tested in the same rat, thus indicating a relative concentration of radioactivity and specific localization of the antibodies. Similar tests performed with antipancreas globulins point to a specific localization of antibodies as shown by the relative concentration of radioactivity in the pancreas. The response of the rat pancreas to the injected specific antiserum was evaluated by blood glucose determinations. A rise of glucose values with a peak up to 250 mg. % was recorded as compared with the upper limit of 135 mg. % in untreated rats or rats treated with normal rabbit serum, or with antiliver immune serum.

Antirickettsial activity of a new synthetic antibiotic. Ludwik Anigstein and Dorothy M. Whitney. From Rickettsial Research Laboratory, The University of Texas Medical Branch.

A new synthetic antibiotic chemically related to chloramphenicol produced by Hoffmann-La Roche, Inc., was tried in experimental spotted fever in guinea pigs. The drug was incorporated in diet up to 2.5%, the daily intake varying from 100 to 500 mg. per day per guinea pig. Treatment of guinea pigs with the drug preceding and following the infection with spotted fever showed a clinical suppression of the disease. This was evidenced either by a complete absence of fever or by abortive attacks of fever of short duration. The recovered guinea pigs when challenged with spotted fever showed complete immunity to reinoculation. The preliminary results indicate rickettsiostatic activity of the new antibiotic, however the effective time-dose relationship has not yet been established.

Comparative Susceptibility to Antibiotics of Organisms Isolated from Air of Hospitals. Frank B. Engley, Jr., and Chin Min Hsiang. The University of Texas Medical Branch, Galveston.

Micrococci (Staphylococci) isolated from routine sampling of air populations in hospital and non-hospital areas have been compared as to sensitivity to antibiotics. One hundred strains from each source were tested for susceptibility by the paper disc

technic to aureomycin, bacitracin, chloromycetin, dihydrostreptomycin, neomycin, terramycin, penicillin, and polymyxin B. Of the antibiotics tested no marked increased sensitivity was noted with bacitracin, chloromycetin, dihydrostreptomycin, and polymyxin B. Strains from hospital air showed a striking decrease in sensitivity to aureomycin and terramycin. The sensitivity data with penicillin revealed a marked division of the staphylococci into two groups with the great percentage of strains much more resistant than the others. The data reveals that the bacterial air population in hospital areas contains greater numbers of organisms more resistant to certain of the more frequently used antibiotics than air of non-hospital areas.

The Antimicrobial Action of Selenium Sulfide Suspension (Selsun). Frank B. Engley, Jr., and Martha Jordan. The University of Texas Medical Branch, Galveston.

A preparation of selenium sulfide (2.5%) containing the anionic surface action agent Nacconol has been tested by the agar plate technic for antimicrobial activity. The test material was placed directly on seeded agar plate surfaces for test. Marked inhibition was noted against the gram positive test organisms; *B. subtilis*, *S. aureus*, *Sarcina lutea*, and *Streptococcus* spp. The gram negative organisms; *Porteus*, *Pseudomonas*, *Klebsiella*, and representative coliforms showed less inhibition. The sensitivity of higher microorganisms was also tested. Six representative *Candida* spp. showed great sensitivity to the preparation. Representative *Microsporum* and *Tricophyton* showed marked sensitivity when planted on plates streaked with the selenium sulfide preparation. Control studies with Nacconol indicate that some of the antimicrobial activity measured by the plate technic is due in part to the surface active agent.

Attempts to Propagate Coxsackie Viruses in Embryonated Eggs. Paul Donaldson, Marjorie Schwab and S. Edward Sulkin. Department of Bacteriology, Southwestern Medical School of The University of Texas, Dallas, Texas.

Extensive trials were made with two Group A strains, a type 4 (Dallas-M.B.) and a type 2 (Fl.) strain. Limited studies were

made with three Group B strains. Seven day embryonated eggs were inoculated via the yolk sac with at least 0.5 ml. of 10 per cent suspensions of infant mouse carcass tissue or yolk sac and chick embyro leg tissue and incubated 6 or 7 days at 37° C.

Four unsuccessful attempts to establish the Dallas-M.B. strain in serial egg passage were made. One of these was made after four alternating passages between embryonated eggs and infant mice and two after six such alternating passages. The other was a serial passage directly from the infant mouse passage virus. The original serial egg passage with the type 2 (Fl.) strain was lost by the sixth consecutive passage and accordingly three alternating passages, as indicated by Huebner, *et al.* (Pub. Heath Rep., 1950, v65, 803), between embryonated eggs and infant mice were initiated with the virus from the third egg passage. Then titrations in infant mice gave evidence of virus proliferation in three of seven consecutive egg passages. The virus was lost in spite of two attempts to continue the series.

Negative results were obtained with the other strains of Coxsackie viruses.

Response to *Vibrio metschnikovii* Vaccine in Irradiated Cancer Patients. Cora L. Gott, David B. Hinds, E. Staten Wynne. Department of Pathology (Division of Experimental Pathology), The University of Texas M. D. Anderson Hospital for Cancer Research, Houston, Texas.

Immune response to *Vibrio metschnikovii* vaccine was studied by the "growth agglutination" technique in 29 individuals undergoing irradiation therapy for malignant neoplastic diseases and 13 non-irradiated cancer patients. Six normal individuals were used for comparative purposes. In the absence of native titers, maximum titers attained in the 3 groups were similar. In cases having native titers, fold increases in titer obtained were not appreciably different among the 3 groups. Intensive analysis of the data failed to reveal any importance of such factors as site or total amount of irradiation, time of irradiation relative to time of injection, or type of neoplasm.

Enzymatic Adaptation of Salmonella Typhimurium to Tartrate. Allen G. Marr and E. Staten Wynne. Department of Plant Sciences, University of Oklahoma, Norman, Oklahoma, and Department of Pathology (Division of Experimental Pathology), The University of Texas M. D. Anderson Hospital for Cancer Research, Houston, Texas.

Decomposition of DL-tartrate by 2 strains of *Salmonella typhimurium* appears to involve an adaptive enzyme system which is formed only under relatively anaerobic conditions. A specific dehydrogenase was demonstrated by the Thunberg technique.

Aeration at 37 C, but not 4 C, rendered adapted cells incapable of attacking tartrate. However, full activity was restored by metabolism of catalytic amounts of glucose or pyruvate, while cysteine and thioglycollate were without effect. Cells grown aerobically on tartrate agar and allowed to metabolize glucose were inactive. These findings suggest that a factor essential for activity of the tartrate decomposing enzyme system is depleted during aerobic metabolism.

Interference Phenomenon Between Human Tumors and a Rat Sarcoma in the Yolk Sac of the Embryonated Chicken Egg. James O. Norman, E. Staten Wynne, and William O. Russell. Department of Pathology (Division of Experimental Pathology), The University of Texas M. D. Anderson Hospital for Cancer Research, Houston, Texas.

A study has been made of the effect of human tumors on the growth of an anaplastic methylcholanthrene-induced rat sarcoma in the yolk sac of the embryonated chicken egg. For various reasons, data with 14 of 29 tumor minces were unsatisfactory. Fourteen of the remaining 15 showed a significant degree of inhibition of rat tumor growth. These included six breast adenocarcinomas, one rectal adenocarcinoma, one papillary thyroid carcinoma, one basal cell carcinoma, one lipoma, 3 squamous carcinomas, and one fibroadenoma of breast. No gross or histologic evidence of growth of human tumors was obtained.

Of eleven tumor filtrates tested, seven yielded usable data and six exhibited interference. These included four adenocarcinomas of human breast, one adenocarcinoma of bovine lung, and one squamous cell carcinoma of bovine eye. The seventh filtrate also showed interference when tumors growing in its presence were passed to a second series of eggs. Similar passages with tumors grown in the presence of minces of human tumors generally showed an increase in the degree of inhibition in passage series.

Of 12 non-neoplastic tissues tested, five yielded satisfactory data but none exhibited detectable interference with rat sarcoma development. Two of these were from the same general anatomical area bearing a carcinoma which did exhibit the interference phenomenon.

The Oncolytic Effect of Viruses in Tissue Culture. Morris Pollard and Robert Bussell, The University of Texas Medical Branch, Galveston, Texas.

Transmissible sarcomas of mice and of rats were propagated in roller tube cultures and then observed following their inoculation with viruses. St. Louis encephalitis virus rapidly destroyed such tumors while neurotropic influenza A virus was slower and less complete in its effect. Fixed rabies virus appeared to stimulate growth of such tumors. Herpes, chick embryo adapted influenza A, and Lansing poliomyelitis viruses had no effect on the tumor growth.

Two malignant tumors from humans were similarly studied: an anaplastic parotid tumor and a malignant abnominal adenocarcinoma demonstrated the same sensitivity to viruses as were manifested by the rodent tumors referred to above.

The Isolation of Induced Mutants of *Serratia marcescens*. Robert P. Williams. Department of Microbiology, Baylor University College of Medicine, Houston.

The techniques of biochemical genetics have been applied to ultraviolet induced mutants of *Serratia marcescens* in the hope that a study of biochemical mutants would yield information concerning the biosynthesis of the bacterial pigment. The me-

dium of Bunting was used for minimal medium and was enriched with enzymatic casein hydrolysate and yeast extract to provide complete medium.

A total of thirteen biochemical mutants have been produced using the techniques of delayed enrichment, limited enrichment, and several variations of the penicillin method. The recent penicillinase method of Adelberg produced no biochemical mutants.

The response of four mutants was determined. They showed growth response to histidine, methionine, tryptophane, thiamine and/or aspartic acid. No attempt has been made as yet to correlate growth response of biochemical mutants with pigment production.

In addition eight "pigment variants" have been isolated. These variants grow equally well on complete or minimal medium but fail to develop pigment on the latter. Their biochemical response has not been determined.

By random isolation of ultraviolet mutants several apparently stable white strains of *S. marcescens* were produced. These have been maintained for further study.

ELECTRON MICROSCOPE STUDIES OF THE VIRUS OF MEASLES (MORBILLI)*

REGINALD L. REAGAN, MARILYN HARMON, WILLIAM DAY, AND A. L. BRUECKNER

Measles is caused by a filtrable virus which attacks man and may be transmitted experimentally to young *Macaca rhesus* monkeys. It has been grown on the chorioallantoic membrane of chicken embryos (Rake and Shaeffer, 1940) and in tissue cultures (Plotz, 1938).

Materials and Methods

Defibrinated venous blood collected from 2 patients with measles at the time of the first appearance of Koplik's spots, fever, cough, conjunctivitis, and rash was obtained through the courtesy of Dr. W. L. Dorrance of the Children's Hospital, Washington, D.C. These 2 patients were bled from the marginal vein, and the defibrinated blood was stored at −20° C. for 2 days before initiating the experiment. Since the virus is quite susceptible to heat, it was kept chilled whenever possible, and all glassware was chilled before being used.

Monkeys *(Macaca rhesus)* weighing from 2 to 3 lbs. were used throughout the experiment. All animals were housed for the period of the experiment in individual cages; and normal, immune, and infected monkeys were kept in separate rooms. Isolation precautions were maintained in handling the animals. The animals were carefully examined each day for development of changes in the skin, eyes, nose, and inside of mouth. Rectal temperatures were taken each morning but no blood was drawn for blood leucocytic examination. The animals were caught in such a manner as to minimize exertion and excitement and then were allowed to relax before the temperatures were taken. A diet consisting of milk, bananas, oranges, and apples was provided throughout the experiment.

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Experimental

Two monkeys, No. 1 and No. 2, were lightly anesthetized with ether and were exposed intranasally with 2 ml. and subcutaneously with 6 ml. of pooled defibrinated blood from the 2 measles cases stated above. Two monkeys, No. 3 and No. 4, were injected with normal defibrinated blood in the same manner. On the 6th day post inoculation the rectal temperature of monkey No. 1 reached 104.0° F., and typical symptoms of measles were noted. Koplik's spots were seen on the buccal mucosa, and a slight rash was noted on the chest and around the axillae. This monkey was bled from the heart for 40 ml. of blood. The monkey survived the bleeding, and daily observations were continued for a period of 19 days. On the 15th day after inoculation, this monkey developed tremors in 1 leg. On the same day, a rash appeared on the face and neck of monkey No. 2, and his temperature reached 104.0° F. Monkeys No. 3 and No. 4 remained normal and on the 6th day after inoculation, monkey No. 3 was bled from the heart for 40 ml. of blood which was used as a normal control.

The infected blood from monkey No. 1 and the normal blood from monkey No. 3 was kept at — 4° C. for 1 hour. The blood specimens were then centrifuged for 5 minutes at 2,000 r.p.m. in a chilled angle centrifuge. The serum was removed from each and filtered through type ST size L3 Seitz filters. The filtrates were then subjected to centrifugation under refrigeration in a Spinco ultracentrifuge for 2½ hours at 44,770 r.p.m. The temperature of the refrigerated outer jacket stayed constant at —10° C. during the centrifugation. After the 2½-hour centrifugation period, the supernatants from the specimens were discarded, and the sediment from each specimen was resuspended in 0.8 ml. of chilled physiological saline. Several drops of each suspension were placed on several parlodion film supports which had been prepared 48 hours previously. After all excess fluid had been removed with small capillary pipettes, the films were dried and shadowed with chromium (Williams and Wyckoff, 1946) at arc tangent 2/10 and examined under the R.C.A. electron microscope, type E.M.U.

The balance of the material of the infected serum was injected intranasally and intravenously into 2 susceptible monkeys

(No. 3 and No. 4) and into 2 monkeys (No. 5 and No. 6) which had been passively immunized against measles by intramuscular injections with 1 ml. of immune serum globulin (Lederle). On the 5th day monkey No. 4 showed a rash on the face and chest, occasional Koplik's spots, and some coryza. Monkey No. 3 developed a rash on the 6th day. Monkeys No. 5 and No. 6 remained normal during a 19-day observation period.

Upon electron microscopic examination of the concentrated suspension from the infected serum, uniform virus-like particles were observed. They were present in practically all of the 2,000 microscopic fields examined. Figures 1A and 1B are electron micrographs showing these virus-like particles. The majority of these particles are spherical in shape and measure 90–100 mu by direct measurement. Irregular contours may be seen on occasional particles as demonstrated by the particle in the lower left-hand corner of figure 1A. This irregularity may be due to the hypertonicity of the suspending fluid during the drying process. In the upper left-hand corner of figure 1B there appear to be 2 particles, one superimposed upon the other. The large irregular masses (figure 1A) appear to be aggregates of particles considerably smaller than those previously described and of undetermined nature.

No virus-like particles were seen in the concentrated normal serum in approximately 2,000 fields examined by electron microscopy.

Summary

Studies by electron microscopy of serum of monkeys infected with the virus of measles show the virus to be round, with occasional particles having irregular contours. The virus particles have a diameter of 90 to 100 mu. These bodies could not be demonstrated in normal monkey serum subjected to the same procedure of concentration and electron microscopic examination. These bodies resemble the virus of influenza described by Stanley (1946). The virus in the concentrated material was infectious for susceptible monkeys but had no effect on monkeys previously immunized against measles with immune serum globulin.

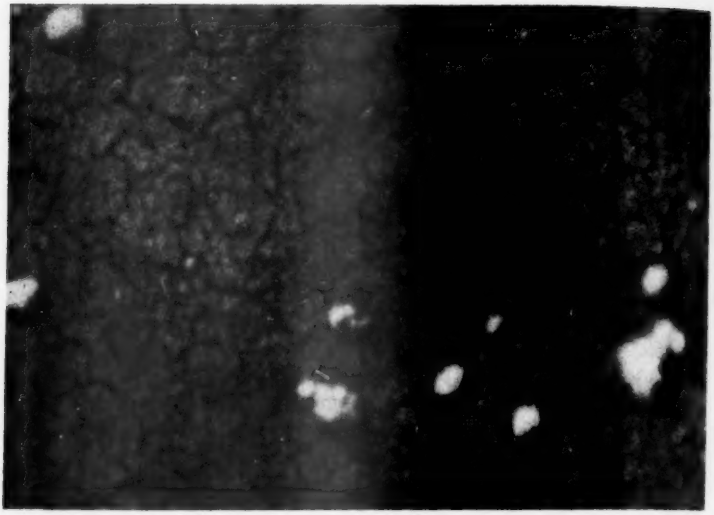

FIG. 1A. Measles virus shadowed with chromium at arc tangent 2/10, X 130,000.

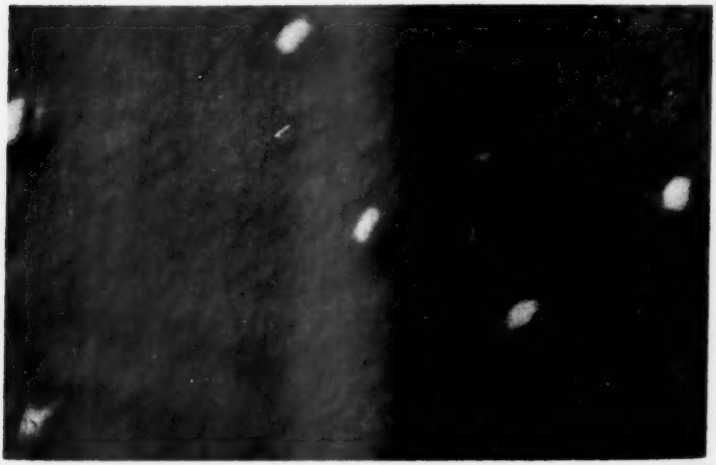

FIG. 1B. Measles virus shadowed with chromium at art tangent 2/10, X 130,000.

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ANEURYSM OF THE THORACIC AORTA

ROBERT ROOE SIMPSON*

Among the great variety of recognized diseases aneurysm of the thoracic aorta can be considered one of the most interesting. Evaluation of the symptoms and clinical signs may allow an immediate tentative diagnosis, because of the accurate anatomical correlation of findings with intrathoracic lesions. On the other hand, cases of intrathoracic aortic aneurysm may be overlooked clinically or even radiologically. Osler (1908) stated, "There is no disease more conducive to clinical humility than aneurysm of the aorta. Mistakes occur with the most careful and the most skillful. Sometimes the diagnosis is beyond our art; more often it is not made because of the carelessness that so easily besets us in our work."

The aneurysm may reach immense size and remain clinically "silent," being detected eventually only by roentgen-ray examination, or necropsy. Even after it is noted by radiological examination, a thorough physical examination may fail to elicit signs suggesting the presence of a lesion. Further, it is important that a large portion of these lesions are not found by radiological examination. In a study by Kampmeier (1938) of 70 cases of thoracic aortic aneurysm coming to necropsy, the lesion was missed by radiological examination in 17 cases (22.6 per cent). The main reasons were: 1.) associated findings obscured the mass; 2.) an incorrect diagnosis was made; 3.) no abnormality was visualized. Therefore, even in those cases readily diagnosed clinically, the difficulty in substantiating the diagnosis may be considerable.

It is the extreme variability of this lesion, primarily its location and size, that makes the clinical evaluation of these cases highly absorbing. In its varying clinical manifestations it may simulate many different diseases.

This paper presents a case with findings typical of both the anteriorly and the posteriorly placed varieties of aortic aneurysm, and in addition, an unusual associated finding.

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Etiology

An aneurysm usually results from some established disease of the arterial wall, such as atheromatous degeneration or syphilis, or, occasionally, bacterial infection. Rarely, an aneurysm arises in a healthy artery as the result of an extension of inflammatory disease from an adjacent organ or of traumatic rupture of the aortic elastic-muscular coat.

Syphilis gives rise to the majority of aneurysms of the thoracic aorta; from 70 to 85 per cent of all aortic aneurysms can be attributed to syphilis. About 92 per cent of aneurysms due to syphilis are in the arch of the thoracic aorta.

In addition to the presence of syphilitic aortitis, with weakening of the vessel wall, the internal strain has an essential part in the production of aneurysm. If the vessel wall is markedly diseased it may be unable to resist the strain of normal blood pressure. The concept has been stated that in many cases of aneurysm, the artery has undergone a severe strain such as occurs with sudden or violent effort, but this latter is questionable, because the onset of syphilitic aneurysm is not acute and no specific episode of elevated blood pressure could be shown to have a part in dilatation of the diseased aorta. Some statistical evidence has been presented which tends to show that more syphilitic aneurysms occur in heavy laborers; however, such an isolated observation has little significance.

Incidence

The incidence of aortic aneurysm found at necropsy varies considerably with different authors. Lucke and Rea (1921) reported an incidence of 2.2 per cent in 12,000 necropsies in Philadelphia covering a period of 49 years; and, in a summary of 160,145 necropsies in different countries (United States, Great Britain, Scandanavian countries, Germany and Austria) there was an overall average incidence of 0.9 per cent. Osler (1908) found an incidence of about 2.9 per cent in his Johns Hopkins series of 2,200. Lemann (1916) in New Orleans found about 3.3 per cent aortic aneurysms in necropsy material. The highest reported incidence is that of Brindley and Schwab (1930) who found aortic aneurysms in 4.36 per cent

of necropsies. The variations in these reported rates of incidence are likely due to varying geographical sites, socioeconomic levels, race and dates of the studies.

The lesion seems to occur less frequently as time goes on and it will perhaps continue to do so with more frequent and adequate diagnosis and treatment of syphilis.

Distribution

There is a preponderance of aortic aneurysm in men with a compiled average of 5.4 to 1. There has been an average race distribution in negroes and whites of 3.3 to 1, respectively. The age range of occurrence of aortic aneurysm is predominantly middle life, 35 to 55 years of age; but they occur in infancy and senility. In comparing reports of age distribution over a period of many years, the age of occurrence seems to be increasing as time passes.

Prognosis

The usual life expectancy after diagnosis is two to three years, but may be less than one week or as long as fifteen years. In all arch aneurysms the duration of life from the onset of symptoms averaged 7.4 months in a report by Kampmeier (1938); but he selected only cases that had a duration of symptoms less than two years. He felt the cases of longer standing were too few to be included for accuracy.

Death is due to rupture internally or externally in most cases, or inanition subsequent to esophageal compression, asphyxia following tracheal compression, or to complications.

Pathogenesis

Generally it has been agreed that aneurysms arise because of two factors, namely, disease of the vessel wall and the pressure of blood against a weakened focus in the vessel wall.

Syphilitic aneurysms are due to a syphilitic inflammation and degeneration of the wall of the aorta. Syphilitic aortitis occurs in from six months to fifty-five years after the primary infection—the average time being twenty years.

The organisms reach the wall of the aorta by way of the lymphatics and vasa vasorum, and produce a syphilitic arteritis. This involves the vasa vasorum with perivascular infiltraton of lymphocytes, plasma cells, and an occasional giant cell. The process extends into the media and causes degeneration and necrosis of the muscle tissue, associated with rupturing of the elastic fibers. At this time there is often thickening of the overlying intima. The degenerated and necrotic areas in the media become replaced by a thin, inelastic cicatrix of fibrous tissue. The weakened, inelastic area of the aortic wall gives way after a time to the constant pounding of the heart or to sudden or prolonged exertion with a rise in blood pressure.

The effects on neighboring structures are, sequentially, adhesion, compression, erosion; and rupture may follow. Lucke and Rea (1923) stated that erosion of any tissue is always accompanied by adhesion to the structure to be eroded, and that commonly the aneurysm has also produced compression.

Boyd (1924) found that in the thoracic aorta about 50 per cent of the aneurysms are in the ascending aorta, 35 per cent in the arch and 15 per cent in the descending aorta. This may be due to the method in which the blood leaves the heart resulting in a relatively high lateral pressure in the ascending aorta, somewhat lower pressure in the arch and still lower pressure in the descending aorta.

Case Report

W. F. L. (No. 149637L) was a seventy-three-year-old white male.

His chief complaints on admission were: 1.) cough for eleven years; 2.) spitting up blood for ten months and 3.) difficulty in swallowing for three weeks. The cough, which was productive of a moderate amount of white frothy sputum, had been present for eleven years, being more severe in the winter and becoming more intense during the past year. Since about 1939 the patient had become increasingly more hoarse. He had been told by his family and friends that his voice had changed somewhat in the past two years. Hemoptysis began about ten months before admission. This consisted of blood streaked sputum being coughed up about twice weekly, with several episodes of about a teaspoonful of blood being present. In the three weeks preceding admission, the patient noted a dysphagia with dry foods. In the past few years the patient had noted a feeling of pressure or fullness in the upper chest anteriorly. The patient said he became short of breath with exertion, but no orthopnea was present.

Upon questioning the patient about perspiration, he stated that his right arm showed more perspiration than his left for the past ten years.

In the patient's past history was revealed a syphilitic infection 40 years before his hospital admission. This infection at the time of occurrence was treated by "a few tablets by mouth." Eleven years ago the patient was told he had, "a chronic heart ailment and a dilated bronchus." At that time he was again treated for syphilis. Five years before admission, he had a "four plus serology" and underwent a series of "several hundred shots, mostly bismuth," for three years until he had two negative and one positive serologic tests. No other past history of significance was given.

The family history was negative for heart disease, tuberculosis, cancer, diabetes mellitis, mental and nervous diseases.

On physical examination the patient was found to be a well developed, fairly well nourished man appearing to be about the age stated. He had a pale color and cyanosis of the lips, had a "brassy" cough, hoarseness and an inspiratory stridor.

The patient's pupils were round, regular and equal, and reacted to light and accommodation. The fundus showed an arteriovenous ratio of one to three, but otherwise was normal.

On examination of his neck venous distention upon reclining was noted. A tracheal tug was felt.

There was a moderate dorsal kyphosis with prominence of the sternal angle, but no other abnormality of the shape of the chest. There was a rhythmical protrusion of the upper sternum synchronous with cardiac systole. The excursion of the chest with respiration was limited, resulting in predominantly abdominal breathing.

Percussion elicited a dullness extending bilaterally about 2 cm. lateral to the manubrium sterni. There was also dullness in both lung bases, more marked on the left. The excursion of the left diaphragm was decreased.

Decreased breath sounds were noted in the left base posteriorly and scattered ronchi were heard throughout the left chest and in both bases.

The P.M.I. was in the fifth intercostal space in the midclavicular line. The heart beat was regular with a rate of eighty per minute. The heart beat was felt over the entire anterior chest. A harsh systolic murmur was heard over the entire anterior chest, but was loudest in the aortic area and was transmitted into the neck. There was a loud slapping aortic second sound. The blood pressure was 140/85 in both arms, and the radial pulses were equal bilaterally.

No masses or tenderness were noted in the abdomen, and the liver, spleen and kidneys were not palpitated.

The examination of the external genitalia showed nothing abnormal. The prostate was moderately enlarged, nodular, and non-tender.

Indirect laryngoscopy was interpreted as showing unilateral incomplete paralysis of the vocal cord on the left.

The hemogram and urine were normal. The only laboratory findings of significance were Wassermann, Kline, Mazzini, and Cardiolipin tests of four plus.

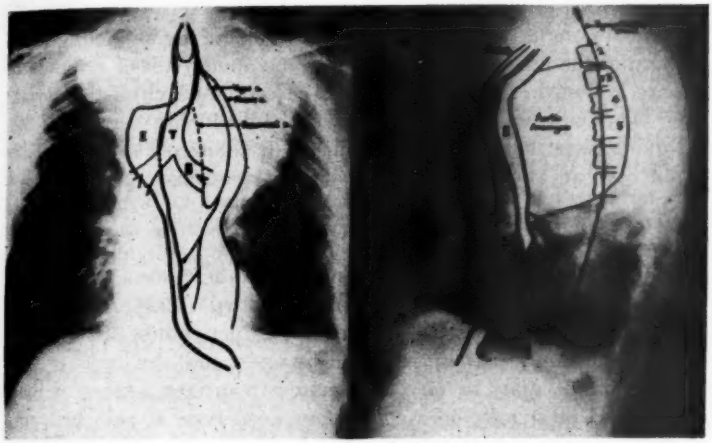

FIGURE 1

The roentgenograms (figure 1) were interpreted as showing a large mass in the posterior mediastinum with calcification in its margins, displacement of the esophagus and trachea anteriorly and to the right, erosion of the anterior portions of the fourth and fifth thoracic vertebrae, calcification in the ascending aorta, and flattening and elevation of the left diaphragm with paradoxical motion of the diaphragm. Both flouroscopy and roentgen kymography failed to reveal pulsations of the mass.

Minor's (starch-iodine) Test showed a relative anhidrosis of the left upper extremity and of the left side of the head and body from the forehead to the level of the fourth rib.

A diagnosis of syphilitic aortitis with a saccular aneurysm of the transverse and descending arch portions of the thoracic aorta was made.

The patient was discharged with symptomatic treatment to minimize his cough.

The patient was followed in the out-patient clinic. He had been seen four months after discharge without progression of findings or new complaints.

Discussion

As there was no pathologist's report to definitely designate the portion of the intrathoracic aorta involved in the above

case, the diagnosis was made on the basis of symptoms, signs and roentgenologic findings.

Thoracic aortic aneurysms have been classified by Broadbent into "aneurysms of signs" and "aneurysms of symptoms." Aneurysms of signs are those occurring in the ascending portion of the arch of the aorta, while the aneurysms of symptoms are located in the transverse and descending arch of the aorta.

This case was an aneurysm of the arch, but seemed to be more posterior by roentgenography. It combined the essentials of both an aneurysm of signs and an aneurysm of symptoms.

Pain

In most cases of aneurysm of the thoracic aorta, pain is at some time a conspicuous symptom. Boyd (1924) has stated that pain was the chief complaint of 29 per cent of 4,000 cases, but that the location is not diagnostic and the type is not specific. It may be no more than a transient feeling of discomfort or it may be a continuous deep type of pain beneath the sternum, in the back, or occasionally in either or both arms. The pain may be that of intercostal neuralgia. The pain may be continuous or it may occur in paroxysms. The episodes of pain may appear as the syndrome of angina pectoris. Erosion of vertebral bodies commonly gives rise to boring continuous pains. Pain is due to compression of structures around the aneurysm.

Dyspnea

Kampmeier (1938) found that dyspnea occurred in 65 per cent of cases of aneurysm of the ascending or transverse arch of the aorta and was generally due to tracheal, bronchial or pulmonary compression. The dyspnea may be so mild as to be disregarded by patients. Boyd (1924) found dyspnea reported as the first or chief complaint in 31 per cent of his collected cases of aneurysm of the thoracic aorta; and severe and steadily increasing dyspnea resulted from compression of the lungs. Mechanical pressure on the trachea and resultant respiratory difficulties are very common.

Several authors refer to a paroxysmal type of dyspnea, of unknown pathogenesis, but rarely seen in the absence of

pressure on the trachea or large bronchi. Hall (1913) states that there are two recognizable types of dyspnea, a paroxysmal dyspnea and a gradually increasing difficulty of breathing, the former probably due to implication of the vagi or recurrent laryngeal nerves, or to compression of nerves that form the pulmonary plexuses; and the latter to direct pressure upon the trachea or bronchial tubes. Tracheal compression usually results from pressure by aneurysm of the transverse arch or of the innominate artery. In most cases of bronchial compression the aneurysm arises from the descending arch of the aorta. Dyspnea may also result from congestion of the lungs, from pressure on the vessels in the root of the lung or from dilatation of the bronchi.

Cough and Hemoptysis

A cough has been noted in about 50 per cent of cases of thoracic aortic aneurysm and is one of the chief complaints in a large number of cases. "Brassy" cough is either absent or non-characteristic in a majority of cases. As a rule it is present when there is involvement of the recurrent laryngeal nerve, which practically restricts its presence to arch aneurysms. When an aneurysm comes in contact with the trachea or bronchus there is always a tendency to cough, therefore, this symptom is frequent with transverse and descending arch aneurysms. Cough and expectoration are manifestations of pressure on the respiratory tract causing irritation and impaired drainage of secretions. Cough with sputum is present in about 20 to 25 per cent of cases. Hemoptysis is found in only a few cases and is usually associated with pressure upon the trachea, bronchus or lung. At times an aneurysm sac eroding into the respiratory tract may ooze blood into the tract for days before rupture occurs. Compression may lead to bronchitis, tracheobronchitis or bronchietasis, with which there may be noted cough, sputum, and hemoptysis. The congestion due to compression of the lung may also lead to blood-streaked sputum. The lung immediately adjacent to an aneurysm may become congested and atelectatic and may eventually undergo fibrosis. Other pulmonary complications consist of pleural effusion, hemothorax, bronchopneumonia, pulmonary abscess, and tuberculosis.

Hoarseness and Stridor

The incidence of hoarseness is especially high in transverse and descending arch aneurysms due to their relationship to the recurrent laryngeal nerve. However, a saccular aneurysm of the ascending arch, pointing upward, may lead to pressure on the right laryngeal nerve; this is a fact not generally appreciated. The left recurrent laryngeal nerve was affected nineteen times and the right two times in a series of thirty-five consecutive cases. Changes in the vocal cords due to pressure on the left recurrent laryngeal nerve were followed by Hall (1913). At first there was increasing paralysis of the abductor muscle, the cord was less vigorous in its movements; later all power of abduction ceased and the cord was found in the midline. Still later the adductor muscles were implicated and the cord was seen motionless in the cadaveric position. The abductor fibers of the recurrent laryngeal nerve succumb to the effects of pressure or disease before the adductor fibers, consequently the vocal cord, at an early stage of the disease, may be in the position of adduction; in this event the voice is practically normal.

Respiratory stridor has been noted in about twelve per cent of 205 patients with aneurysm of the transverse arch. Such a finding is much less frequent in cases with aneurysm of the ascending or descending arch. The stridor is due to compression of the trachea or it may be due to involvement of the recurrent laryngeal nerve.

Dysphagia

Intrathoracic aneurysms of the aorta may be mistaken for intrinsic disease of the esophagus since it may produce dysphagia, anorexia, nausea, vomiting, weight loss and hemetemesis. Dsyphagia is found most often in the transverse arch aneurysms because of the intimate relationship to and compression of the esophagus.

Anhidrosis

In reviewing the literature upon aortic aneurysm, no report was found of a case showing relative anhidrosis in the absence of other features of Horner's syndrome or inequality of the

pupils. The case presented herein displayed the finding of relative anhidrosis of the left side of the face, neck and chest and left arm. Apparently the aneurysm had encroached upon the sympathetic trunk below the region that transmits the preganglionic fibers from the first and possibly the second thoracic roots. The absence of any anisocoria or Horner's syndrome would indicate that the sympathetic preganglionic fibers supplying the eye arise from a higher level than those supplying the sweat glands of the head and neck. This seems to be borne out by some recent work by Thompson, Brose, and Smithwick (1950). After removal of the second and third dorsal nerves in the usual sympathectomy, they found the face, arms, hands, and upper thorax generally to be completely anhidrotic and Horner's syndrome absent. They also found that removal of the first dorsal ganglion generally resulted in Horner's syndrome. It is also of interest that in some of their patients in whom the first dorsal ganglion was resected, Horner's syndrome was not observed. This was interpreted as meaning that preganglionic fibers to the pupils in these patients arose above the first dorsal ganglion, namely, in the inferior cervical or above. Ray, Hinsey, and Geohegan (1943) have shown by direct stimulation of anterior roots during laminectomy that the preganglionic innervation to the pupil travels through one or more roots between the inferior cervical and the fourth dorsal ganglion.

Venous Distention

Pressure symptoms and signs include those of interference with the superior vena cava, as exemplified by jugular distention. Distended jugular veins do not pulsate in cases of aneurysm, a fact which distinguishes aneurysm from failing compensation.

Tracheal Tug

In diagnosing thoracic aortic aneurysm, Oliver (1878) first described tracheal tug and the method of eliciting this finding. When it is elicited, it generally indicates an aneurysm of either the ascending or first part of the transverse portion of

the arch. He states that the act of examination will increase laryngeal distress should this accompany the disease.

"The process is as follows: Place the patient in the erect position, and direct him to close his mouth and elevate his chin to the fullest extent, then grasp the cricoid cartilage between the finger and the thumb, and use gentle upward pressure on it, when, if dilatation or aneurysm exist, the pulsation of the aorta will be distinctly felt transmitted through the trachea to the hand."

Palpatory Signs

Three characteristic signs of aneurysm are a mass, an expansile pulsation, and a thrill over the mass.

Some observers rank diastolic shock as the most important single sign of aneurysm; if not that, it is one of the most conclusive signs.

Whether or not expansile pulsation is present depends upon the amount of filling of the aneurysmal sac with clotted blood. It may be difficult or impossible to distinguish active pulsation from that transmitted from a normal aorta through a mass.

A thrill is present in less than 50 per cent of cases according to McSwain (1950). The thrill is usually systolic in time, rarely double or diastolic. If the sac contains much clotted blood, and especially if the anterior portion of the sac is filled, the thrill is usually absent.

Percussion Signs

In certain hands percussion seems to yield earlier information of an intrathoracic growth than any other method of physical examination; Lemann (1916) believes that his percussion findings are well founded. (This includes percussion of the manubrial region, the first, second, and third intercostal spaces and the vertebral column.) If an aneurysm is present, the size may be approximately determined by the extent of the area of relative dullness.

Auscultatory Signs

A murmur, usually systolic in time, is present in the majority of aneurysms. The murmur may be diastolic in time, espe-

cially if the first portion of the arch is involved or a to-and-fro murmur may be present. Probably the most significant auscultatory sign is the presence of a ringing and sharply accentuated second aortic sound. This is coincident with the diastolic shock.

Roentgenography

No examination of a suspected aneurysm or other mediastinal affection is complete without the use of this method of examination. Even in cases in which the symptoms and physical signs appear diagnostic, roentgenography aids in determining the extent and character of the aneurysm. The esophagus and trachea are usually displaced anteriorly and to the right; in order to displace the esophagus anteriorly and to the left an aneurysm must be at the diaphragmatic hiatus. Calcifications of the margins of the aneurysmal sac are often seen in long standing aneurysms. Calcification in the ascending aorta appears to be typical of syphilitic aortitis.

It is well known that a thick walled aneurysm or one filled with clotted blood may show no pulsation by roentgen examination.

Erosion of bone adjacent to the aneurysm is not an uncommon occurrence. It seems to take place without demonstrable compression. D'Istria (1930) in a summary of observations in five aneurysms with vertebral erosion submitted the following radiographic diagnostic points: 1.) excavation of the spinal column; 2.) preservation of discs; 3.) more erosion of spongy centrum than of the upper and lower laminae, and 4.) erosion not uniform in all parts.

The flattening of the left diaphragm with some obliteration of the costophrenic angle observed in the case presented may be due to phrenic nerve involvement on that side and/or pleural complications that arise as a result of extension of an infection from an underlying pulmonary lesion. The questionable paradoxical motion of the diaphragm alluded to previously may have been due to unilateral phrenic paralysis.

Summary

A case of aneurysms of the thoracic aorta is reviewed which is unusual in that the patient showed relative anhidrosis of

one side of the face, neck, and chest down to the level of the fourth rib and arm, without evidence of anisocoria or other features of Horner's syndrome.

Nearly one fourth of aneurysms of the thoracic aorta may be overlooked by roentgenologic examination.

Analysis of many series indicate that aortic aneurysm is decreasing in incidence, perhaps due to earlier and more adequate treatment of syphilis, and it appears that the age at the time of diagnosis is increasing.

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HEMODYNAMIC CHANGES RESULTING FROM INSERTION OF A ROTAMETER IN THE VENOUS CIRCULATION OF A DOG*

EDWIN L. SMITH, RUSSELL A. HUGGINS, ROYAL W. RANDALL, AND GEANE A. JEFFERY

A large rotameter has been used in several laboratories to measure either the total venous return or, more recently, the cardiac output (1, 2, 3, 4). A large rotameter has been used in this laboratory for measuring total venous return in an extensive series of experiments, and we have been impressed with the rapid deterioration of the animals after its insertion. Although the dog may live from 2–4 hours, during a large portion of this time the blood pressure and flow are at very low levels. Further, even during the period in which the blood pressure is at or approaches control levels the blood flow is definitely below that which would be expected on the basis of an intact animal of the same size or one with an open chest. Data are presented in this paper describing the circulatory collapse and various attempts to determine what factors are involved in its development.

Methods

Arterial pressure was measured with a Hamilton manometer through a sound passed down the left carotid to the aorta and venous pressures from the superior and inferior venae cavae, right auricle, and femoral vein with water manometers. The table top was used as the zero for the manometers. The technique used for inserting the rotameter has been described in a previous paper (5). In most instances morphine sulfate, 10 mg./kg. subcutaneously followed by sodium pentobarbital 10 mg./kg. intravenously, was used as the anesthetic; occasionally, sodium barbital 250–300 mg./kg. was used. Chlorazol

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fast pink, 80 mg./kg. or heparin 10 mg./kg. or sometimes a combination of the two, was used as the anticoagulant. In the most recent dogs, Paratol "A" has been tried as the anticoagulant, and we have found it much superior to Chlorazol fast pink and in certain respects preferable to heparin.

Results

The first procedure in inserting the rotameter was to clamp the auricular appendage, make an incision, insert and tie the input cannula. This procedure was accompanied by marked cardioacceleration, irregular rhythms with extra systoles and dropped beats. After tying the cannula, the heart rate slowed to an average of 173 per minute and remained elevated above the control level of 127 per minute. The superior vena cava was occluded and a glass cannula inserted. Flow from the superior vena cava was re-established through the rotameter within 30–60 seconds after the occlusion. During the occlusion, the superior vena cava pressure rose an average of 76% and on release, the pressure fell rapidly toward control levels, usually remaining slightly above it for a variable period of time. The arterial blood pressure fell during the occlusion and on release usually returned to within the control levels.

The same procedure was used on the inferior vena cava except that the period of occlusion was usually between 60–120 seconds, but during this period the heart was receiving blood from the superior vena cava. The blood pressure again fell during insertion of the rotameter and on release of the occlusion, in most instances, tended to increase by a variable amount over a 5 to 10 minute period. The average blood pressure for all dogs at this point was 82/60. There were occasional instances of the blood pressure returning to the control level. The inferior vena cava pressure rose an average of 37 mm. H_2O during the occlusion and on release fell toward control levels.

With the insertion of the rotameter, after a variable period of time but usually not more than 30–40 minutes, the mean blood pressure and venous pressures began to decrease. This decrease continued until the mean blood pressure stabilized between 30–40 mm. Hg., at which time the venous pressure had

also fallen to its lowest level. In this state, the dog may continue to live for 2–3 hours with little change in the measured circulatory events. Figure 1 shows the typical sequence of these events after the rotameter is inserted.

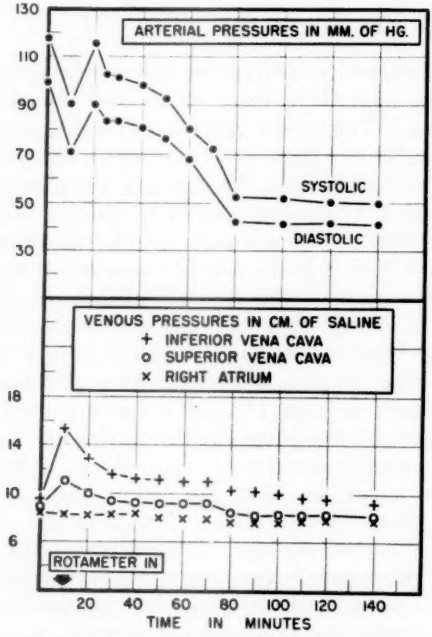

FIG. 1. The sequence of circulatory events following the insertion of a rotameter.

This relatively prompt decline in arterial blood pressure suggested that one or more of the following factors might be involved. One, the operative procedures were too drastic and caused the circulatory collapse; two, the auricular cannula interferes in some manner with the heart so that a central flow failure results; three, the cannulae, tubing, and rotameter represent a resistance too large to allow an adequate venous return; and four, some toxic substance is formed when the blood flows through a circuit of rubber tubing and glass (6).

That the operative procedures, including temporary occlusion of the superior and inferior venae cavae, are not involved, is indicated by Figure 2, which is a record of 1 of 6 dogs whose chest was opened and all techniques duplicated, except the actual insertion of the rotameter. These dogs have been carried for as long as 4.5 hours without sign of blood pressure

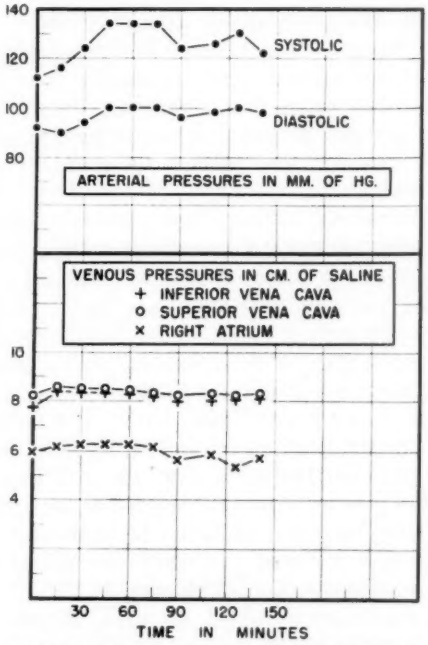

FIG. 2. The sequence of circulatory events in an open chest dog immediately after the sham insertion of a rotameter.

deterioration. Also in 3 dogs, only the auricular cannula was inserted, and the chief response was an increase in heart rate with no evidence of cardiovascular deterioration. The anesthetics and the anticoagulants used were varied with no evidence that they influenced the course of the circulatory failure.

Examination of a curve published by Gregg *et al.* (7) for a large rotameter would indicate that comparatively little resistance should be offered by the instrument. Their curve shows that it required about 1 mm. of Hg. to raise the float and at a flow of 800 cc./min. the pressure loss was about 1.2 mm. Hg. However, these data presumably do not include the necessary tubing and cannulae which are necessary to connect the rotameter to the dog. These could obviously make a very great difference in the resistance of the unit as a whole. A series of calibrations (Figures 3 and 4) were done, including some with the rotameter, tubing and cannulae exactly as it was used in the dog. The data show that a pressure of 1.4 mm. Hg. is necessary to elevate the float, a figure in good agreement with Gregg *et al.* (7). This means that at the common rate of blood flow obtained when the dogs are in good condition (800 cc./min. with an occasional dog having a flow rate up to 1,200 cc./min.) there is a pressure loss of 4.8 to approximately 9 mm. Hg.

If the rotameter is removed from the circuit, leaving only cannulae and the tubing, the pressure loss at any flow was reduced approximately 1.3 mm. of Hg. The data also indicate that the greatest part of the resistance in the rotameter circuit lies in the tubing and cannulae at rates of flow commonly found in this type of preparation. The cannulae represented the greatest part of this resistance and because with our method two cannulae were used, one each for the superior vena cava and inferior vena cava instead of the single cannula used by Shipley (2), his cannula was duplicated and tubing arrangement approximated. No significant difference was observed in the calibration curves (Figure 3).

Reducing the length of tubing and using slightly larger cannulae reduces the pressure loss to 2.4 mm. Hg. at a flow of 800 cc./min. However, the larger cannulae increased the difficulty and the time required to insert them, and the circulatory response was the same despite the reduced resistance.

In addition to the other pressure measurements made, the pressure above the float was determined, the pressures then could be divided into two portions: one, between the input and the inside of the rotameter; and, two, between the inside of the rotameter through the output cannula. At a flow of

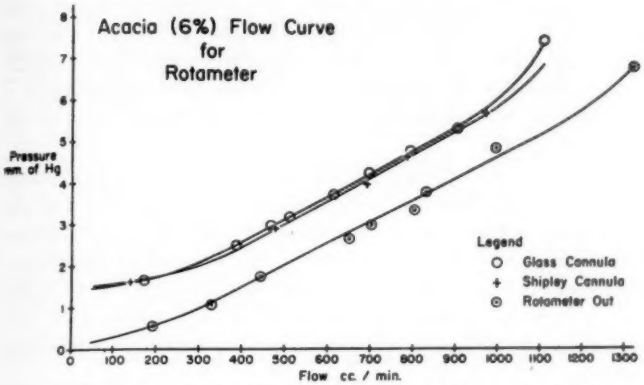

FIG. 3. The pressure loss through a rotameter, tubing and cannulae.

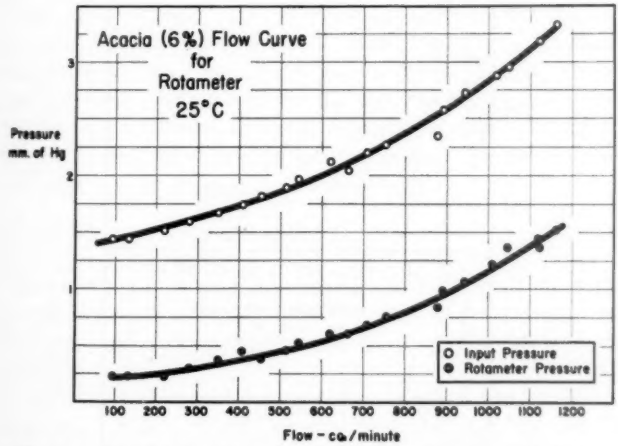

FIG. 4. Shows the reduction in pressure loss found with larger tubing and cannulae. The top curve shows the total pressure loss of the rotameter, tubing and cannulae. The distance between the bottom curve and the top one gives the pressure loss from the point of input to a point inside the rotameter. The distance from the base line to the bottom curve gives the pressure loss from inside the rotameter to the tip of the output cannula.

100 cc./min. 15% of the pressure loss occurred in moving the fluid out of the rotameter (Figure 4) and at 800 cc./min. the percentage increases to 35. These figures suggest that a significant lowering of the pressure loss at high flows would occur if the diameter of the outflow spout was increased or a second outlet attached.

The resistance offered by the rotameter and the necessary tubing and cannulae did not appear to be of the order that the dog should not be able to overcome; to test this point further, the usual procedure was followed, but instead of inserting a rotameter the inferior vena cava was constricted to either 5 or 3 mm. in 16 dogs by the method of Hwang *et al.* (8). In 4 dogs the superior vena cava was constricted in the same manner.

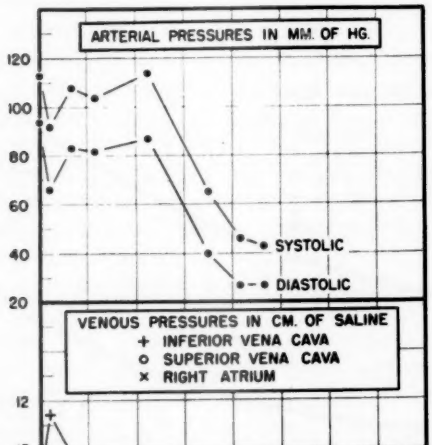

FIG. 5. The sequence of circulatory events following constriction of the inferior vena cava above the diaphragm.

Constriction of the inferior vena cava to 3 mm. resulted in a circulatory failure much like that occurring with the rotameter within 30–60 minutes. When the constriction was 5 mm. (Figure 5), the end result was the same, but the period before the circulatory collapse occurred was more variable, ranging from 30 minutes to 3 hours. Constriction of the superior vena cava in the same manner did not result in circulatory collapse in 4 dogs.

Attempts were made, by giving transfusions from donor dogs at various times during insertion of the rotameter or after it was in, to prevent the development of circulatory failure or to reverse it after failure developed. Blood pressure and flow increased during the transfusion, but within 10–50 minutes the flow and pressure had decreased to the original level. On one occasion, a 20 kg. dog was bled into a 10 kg. dog with only temporary postponement of failure.

The fall in blood pressure does not seem to be the result of a peripheral collapse, as calculation of the vasomotor resistance shows that it remains within control limits. This was also true of the dogs in which the inferior vena cava was constricted to 3 or 5 mm. and total blood flow determined by the direct Fick method. Therefore, in these dogs the mean pressure and cardiac output decline proportionally.

Discussion

The circulatory course followed by these dogs is reminiscent of experiments reported by Wegria *et al.* (9) using a heart-lung-dog preparation in which the animals went rapidly into an irreversible circulatory failure. They refer to this type of failure as "fulminant failure." The principal difference between the circulatory data of their experiments and ours is that their animals showed a precipitous decline in peripheral resistance, whereas in ours the peripheral resistance fluctuated but showed no evidence of changing significantly.

Our data, contrary to that of Stamler *et al.* (10), show that a constriction of 5 to 3 mm. placed on the thoracic inferior vena cava results in rapid circulatory failure. Our experiments were considerably more drastic than those of Stamler, for their experiments were chronic but ours were performed on open chest

animals which had received heavy doses of an anticoagulant. While it is true that these various precedures, including insertions of needles for measuring venous pressure and anticoagulants, did not result in any measured deterioration of the control dogs, it seems possible that, between the operative procedures and drugs used, the animals were at a circulatory threshold over which they could be precipitated into irreversible failure.

Therefore, it is possible to interpret the circulatory collapse occurring on insertion of the rotameter either as the result of additional insults to the circulatory system attendant upon its insertion or to the added resistance to flow offered by the rotameter. The close resemblance between the circulatory events and their timing when the effect of simple constriction is compared to the rotameter suggest that the most likely explanation is the increased resistance offered by this instrument.

The amount of resistance to the flow of blood offered by the rotameter would seem to be within the range that the dog should be able to overcome by an elevation of its venous pressure. It is true that the venous pressure is somewhat elevated after insertion of the rotameter but only for a relatively short period of time and usually not enough to overcome the pressure loss through the rotameter to provide the dog with an adequate venous return. Numerous experiments have been tried in attempting to explain the inability of the dog to increase his venous pressure, all with negative results at the present time.

It would appear, from the results reported by Seely *et al.* (3) and Wegria *et al.* (4) that insertion of the rotameter into the pulmonary circulation does not precipitate the circulatory collapse occurring when it is inserted into the venous system. By this method the right heart or the lung reservoir may serve as a buffer preventing the venous deterioration that occurs when the rotameter is inserted directly into the venous system.

Summary

Insertion of a rotameter in the venous circulation of dogs results in a rapid circulatory collapse, with a mean arterial pressure between 30–40 mm. Hg. with low venous pressures but no evidence of loss of peripheral resistance. The circulatory failure

cannot be prevented by massive transfusions given before, during, or after insertion of the rotameter. Dogs are unable to raise and maintain venous pressure at a level high enough to overcome the resistance offered by the rotameter.

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SERIAL NEEDLE BIOPSY OF THE LIVER: A CASE REPORT*

CHARLES T. STONE, JR. AND FRANK M. TOWNSEND

The following case is of interest because: (1) it represents an extended series of consecutive liver biopsies; (2) it allows unusually close comparison of pathologic specimens with the laboratory data and clinical course of the patient; and (3) it reiterates the lack of correlation between pathology of the liver and liver function tests.

J. W. H. (John Sealy Hospital No. 7280-F), a 47-year-old white divorced merchant mariner, was brought by the local police to the hospital on June 22, 1949, because he was intoxicated and obviously quite ill. Intravenous fluids soon revived him to the point where he could speak; however, it was then apparent he had been intoxicated for some time and was disoriented as to time, place, events, and persons. He was unable to give any coherent history.

Physical examination revealed a dehydrated, confused, dirty adult white male, whose pulse was 108, blood pressure 86/50, and oral temperature 101° F. Examination of the head and neck, including the ocular fundi, revealed nothing unusual. The lungs were clear to percussion and auscultation. The heart was normal size; there was a grade 1 apical systolic murmur and a sinus rhythm. The abdomen was flaccid. A large smooth, hard liver could be palpated 8 cms. below the right costal margin in the midclavicular line. There was no ascitic fluid, and the spleen was not enlarged. The remainder of the physical examination was within normal limits.

Refer to Fig. I for detailed results of the patient's laboratory studies. He was acutely azotemic at the time of admission, but under adequate hydration his kidney function reverted to normal in two weeks.

A psychiatric consultant made the diagnosis of Korsakoff's Syndrome. Clinically the patient also had (1) portal cirrhosis and (2) malnutrition.

The patient was placed on gastrostomy feedings immediately upon admission and was fed in this fashion for twelve days, at which time he was able to feed himself. During this interval his ancillary therapy consisted of two multivitamin capsules daily, four tablets of brewers' yeast three times a day, methionine 0.5 gms. three times a day, 100 mg. nicotinic acid three times a day, syrup of choline 4.0 cc. (1 gm.) four times a day, plus high protein, high carbohydrate and low fat diet. Although his appearance and laboratory studies improved considerably, his mental status remained essentially unchanged.

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FIG. 1

Serial Needle Biopsy of the Liver
(Case Report JWH, JSH #7280F)

	6-22-49	7-6-49	7-11-49	7-22-49	8-9-49	10-21-49	5-11-50	5-18-50
NPN	121	30						
Cholesterol and esters					242 189	233 152	369 148	415 282
Total Serum Proteins	5.80	4.16	7.24			8.20	6.00	6.7
Alb.	3.68	2.16	3.78			4.88	4.3	5.1
Glob.	2.12	2.00	3.46			3.32	1.7	1.6
Serum Bilirubin Direct	0.8			Neg.		Neg.	Neg.	
Total	1.3			0.26		.07	.62	
5 mins		50		70		50	65	
B.S.P. 30 "		20		15		*L10	*L10	
45 mins.		20		10		*L10	*L10	
5 mg. per Kg. of body weight								
Ceph. Flocc. 24H	+		0			+	0	0
48H	++		0			+	0	0
Prothrombin Activity		100%				100%	100%	
Hippuric Acid				.37				
†Alk. Phosphatase				2.72		2.78	1.67	
Hemoglobin: gms.	14.6	9.7			13.2		13.8	13.4
Urinalysis	N	N	N	N	N	N	N	N
Biopsy Number		1	2	3	6	16	18	19

*L = Less than.
†Bodansky units.

On July 6, 1949, the first needle biopsy of the liver was done, using the short Vim-Silverman needle via the abdominal approach. The patient was prepared in the usual manner and 75 mg. of Demerol were given hypodermically shortly before the procedure was carried out. From then on his liver was biopsied weekly, on as nearly as possible the same day, for seventeen consecutive weeks. The first thirteen biopsies were subcostal

in location and the last four were transpleural. For the transpleural route the Hoffbauer modification of the Silverman needle was used. From the pathologist's reports it can be seen that, although the patient's condition improved with return to normal size of the liver, the pathologic changes in the liver remained essentially the same.

He was finally discharged from the hospital on October 25, 1949, at which time his mental status had improved moderately and his physical condition was clinically normal.

The patient was not seen again until May 10, 1950, at which time he was again brought by the police to the Emergency Room because of "convulsions." It was obvious that his dietary and general hygienic habits had not improved in the interval. He had again become malnourished and dehydrated.

Physical examination revealed the oral temperature to be 99.4° F., pulse 62, blood pressure 124/78. The liver edge could be palpated 1–2 cms. below the right costal margin with deep inspiration. Refer to Fig. I for liver function tests upon re-admission. During this hospital stay additional biopsies were done on the following dates: May 11, May 18, May 26, May 31, and July 28, 1950. His general condition again improved under a therapeutic regimen identical with the previous one, and he was discharged August 21, 1950.

Pathologist's Description

An objective study of the entire series of needle biopsies of the liver in this case shows essentially the same findings in each of the biopsies. The sections are characterized by an increase in connective tissue in and about the portal spaces with degeneration and regeneration of liver cord cells adjacent to the connective tissue. Within the fibrous tissue there is an infiltration of leukocytes and mononuclear cells. In some instances the liver cells are slightly vacuolated; however, the changes of fatty metamorphosis are not particularly conspicuous in any of the sections studied. This pattern persists, as pointed out, throughout each of the sections; and it is impossible to distinguish the first biopsy from later piopsies. A study of the last needle biopsy of the liver shows a normal liver architecture with no increase in fibrous connective tissue. Wilder's and other special stains designed to show connective tissue fail to reveal any increase. This, it must be emphasized, is the only one of the biopsies that is not consistent with the diagnosis of portal cirrhosis.

Comment

This patient received a total of twenty-two needle biopsies of the liver. Thirteen were made through the subcostal route and the remaining nine biopsies were via the transpleural approach. None of these biopsies caused the patient any more than minor discomfort, and there was not a single instance of any of the sequela which are occasionally the aftermath of needle biopsies of the liver. This case is unusual in that one biopsy was carried out each week for seventeen consecutive weeks. We are not familiar with any other such large number of consecutive biopsies at regularly spaced intervals. At his first hospital admission his liver was moderately enlarged. During his hospital stay the hepar became progressively smaller so that after a time (thirteen biopsies covering a period of three months) it became necessary to resort to the transthoracic route to secure biopsy material.

In the past several years a great deal of comment has appeared recommending the high protein, high carbohydrate diets in the treatment of chronic liver disease. In this man's case this type of therapy was started as soon as he was admitted to the hospital, and it was continued uninterruptedly for slightly over four months. During this interval he received, in addition to the diet, massive vitamin supplements, choline and methionine. Although his liver decreased in size, histopathologic changes remained essentially the same. A small amount of fat, which was present in the earlier specimens, soon disappeared. It should be noted in passing that at no time did this patient accumulate any ascitic fluid.

The only liver function tests which were definitely abnormal in the early stages of his illness were the serum bilirubin and the bromsulfalein excretion test. By the end of the first month the entire panel of liver function tests had returned to normal; thus, in this case, there were pathologic and clinical evidences of serious damage to the liver which all of the available liver function tests failed to accurately portray. It is noteworthy that in the six-month interval between his discharge from the hospital and subsequent reentry, he reverted to his habits of excessive alcoholic intake and poor nutrition; notwithstanding, upon readmission, his liver function tests were still quite normal. Liver

biopsy at this time revealed the persistence of cirrhosis of the liver.

A rather unusual and unexpected occurrence took place in a two months' span between the twenty-first and twenty-second biopsies in that the twenty-second specimen was reported as normal liver with no evidence of cirrhosis. We have no logical explanation for the relatively sudden change from a picture of cirrhosis to one of normal histology; however, reference is made to the study of Waldstein and Szanto. These authors found on the study of needle biopsies from cadavers that alteration in the lobular pattern, scarring and inflammatory changes showed significant dissimilarity in the amount of alteration in ten percent of the specimens studied.

Summary

A case has been presented in which the patient received 22 needle biopsies of the liver. Our results demonstrate poor correlation between liver function tests and pathological change in the liver. Serial needle biopsy of the liver is thought to be a practical and feasible means of following the course of chronic liver disease. In selected cases extended serial biopsy study is possible.

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An objective study of the entire series of needle biopsies of the liver in this case shows essentially the same histology in each, with the exception of the last biopsy performed on July 28, 1950. This last biopsy is essentially normal. All of the previous biopsies show a pattern that is indistinguishable from section to section.

Figure No. 2 is taken from a biopsy performed on May 18, 1950, and illustrates this typical pattern. There is an increase in fibrous tissue separating the liver parenchyma into a lobular pattern. The portal spaces show an increase in fibrous tissue with an infiltration of monocytes and lymphocytes. Many of the liver cord cells show vacuolization of their cytoplasms which is consistent with fatty metamorphosis.

In the last biopsy performed July 28, 1950, there are a few scattered mononuclear cells. However, there is no increase in the fibrous tissues and the liver architecture is essentially normal. The liver cells are normal. This biopsy is represented by Figure No. 3.

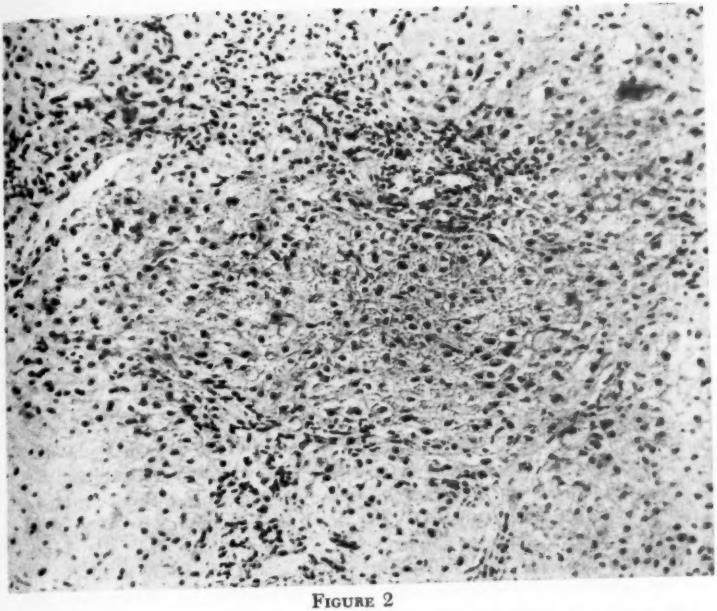

FIGURE 2

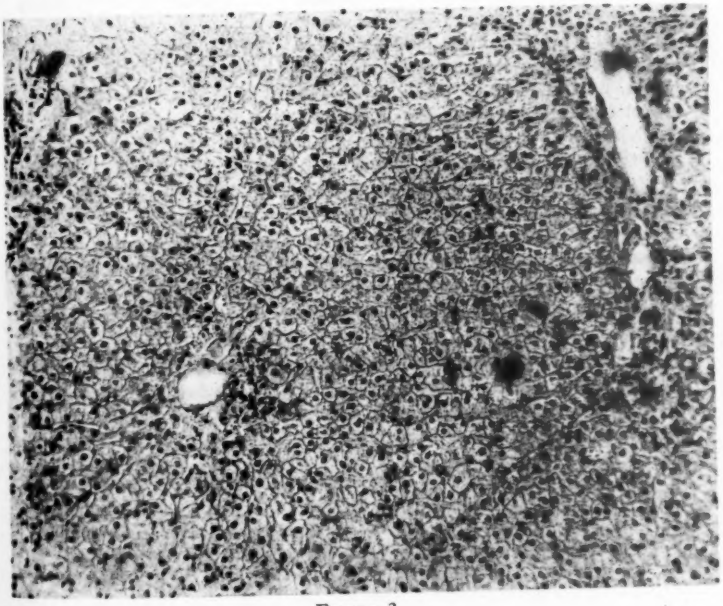

FIGURE 3

SYMPOSIUM ON PRE-PROFESSIONAL TRAINING FOR THE HEALTH TEAM

The University of Texas Medical Branch was host on May 15–17, 1952, to a conference called by Dean D. Bailey Calvin to consider pre-professional health education. This was held under the joint sponsorship of the Texas Academy of Science, the National Honorary Pre-medical Society, and The University of Texas Medical Branch. The conference was featured by a series of general discussion: (1) liaison between colleges and professional schools in relation to health training; (2) the pre-professional health curriculum, and (3) the advisory method in the educational guidance of the student in the pre-professional health training.

The symposium was opened by an address by Chauncey D. Leake, Vice-President of The University of Texas for the Medical Branch, "Education for the Health Team." Further addresses were given by Elizabeth Bixler, Dean of the School of Nursing of Yale University, and President of the Association of Collegiate Schools of Nursing, "Nursing Education in the Health Crisis"; Sumter S. Arnim of The University of Texas Dental Branch, Houston, "The College Curriculum and Dental Education"; F. H. Arestad, Secretary, Council on Medical Education and Hospitals of the American Medical Association, "Training and Utilization of Technical Personnel"; Herman G. Weiskotten, Dean Emeritus, Syracuse University School of Medicine, Chairman, Council on Medical Education and Hospitals of the American Medical Association, "Adventures in Pre-professional Health Education"; J. E. Markee, Professor of Anatomy, Duke University School of Medicine, Durham, North Carolina, "Techniques of Selection of Candidates for Admission to Medical Schools"; Willis G. Hewatt, Professor of Biology, Texas Christian University, Fort Worth, President, Texas Academy of Science, "Experiences in pre-professional Health Education"; John M. Stalnaker, Director of Studies, Association of American Medical Colleges, "Pre-professional Health Education and Professional Medical School Performance"; Hugh E. Setterfield, Professor of Anatomy, Ohio State University School of Medicine, Columbus, President, National Pre-medical Honor Society, "The College and the Pre-professional Health Students."

The conference emphasized the developing opportunities for effective service in the auxiliary health fields. Agreement was reached on the necessity for maintenance of high scholastic standards for admission to study in any of the health fields.

THE EXPANDING TRADITION OF HEALTH SERVICE*

CHAUNCEY D. LEAKE

Vast extensions loom in health services. Thrilling indeed to young people may be the visions of satisfying careers in health affairs, if they can appreciate the logical possibilities of fruitful and helpful application of current knowledge to the promotion of abundant good health for our citizens.

Four phases are apparent in the tradition of the health services. These may be labelled as (1) primitive care and comfort for sick and injured by relatives, friends, and priests; (2) professional management of the diseased and maimed by professionally trained physicians; (3) prevention of disease by individual immunization and by social control of sources of infection or deficiency, and (4) promotion of long, healthy and satisfying living.

Primitive Medical Care

The great tradition of health service began with the elemental succor given to a sick person by friends, relatives or priests who were immediately at hand. This is primitive medical care. From the position of what we now call psychosomatic medicine, it is still highly effective. Kindly care and comfort are genuinely helpful in any sickness or injury. Cheerful trivialities of conventional talk, and the encouraging feel of a friendly hand, offer hope, which remains the best doctor of all. Help in performing the necessary functions of living in eating, drinking, and evacuating is direct support, instinctively realized, toward getting well.

Surprising survivals are to be noted of the extensive traditional lore of this primitive medical service, accumulated by grandmotherly wisdom, and passed along sly mouth to eager ear, for centuries, always semi-secretive, semi-magical, and awesomely

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*Presented at Ohio State University Health Center, Columbus, the University of Alabama Medical Center, Birmingham, Southwestern Medical School of The University of Texas, and the Pre-professional Health Conference at the University of Texas Medical Branch, Galveston.

authoritative. Amazing is the continued publication today of the mysteriously anonymous mediaeval compilation called "The Secrets of Aristotle." A delightful account of such primitive and basic medical service occurring in Columbus, Ohio, is given in *The New Yorker*, in James Thurber's daguerreotype of Margery Albright, healer, midwife, scorner of men and doctors, and sterling character withal. Most of us have had a grandmother or great aunt like her.

The chief danger in this primitive care of the sick is to do too much. Sick people have an amazing tendency to get well regardless of whatever is done for them. All eventually die, of course. Meanwhile, however, each have many episodes of sickness, from any one of which recovery may occur. Hippocratic medicine in ancient Greece took the most significant steps in establishing the effective tradition of medical service by recognizing clearly these two factors in primitive familial care of the sick. *Primum non nocere* was the watch-word, as the Latinists undertok to maintain Hippocratic principles—first do no harm! *Vis medicatrix naturae* was the phrase used to emphasize the force of natural healing.

Failure to appreciate these two essential aspects of primitive elemental care of the sick has been responsible for the growth of that enormous tangle of false procedure, mistaken theory, fraud, quackery, and cultism, through which professional medicine has had to make its painful way. This has resulted from the operation of the basic logical fallacy, into the trap of which all of us are prone to fall. The confusion of mere sequence of events with a cause-and-effect relation is common to all people at all times. We all tend to think post *hoc ergo propter hoc*. Our friend is sick; we give him a medicine; he gets well—and therefore, we think the medicine (or whatever else we may do for him) has cured him. It is too seldom appreciated that the chance is large that he would have gotten well anyway. It is too often the urgent wish that he would get well, which focuses attention on the hope that whatever we do will make him better. Thus, a new drug is introduced, a new theory of disease is developed, a new medical cult established.

Painful and slow is the disillusionment inevitable in the realization of the significance of the Hippocratic critique of primitive

medical care. It is a wholesome experience, however, even for the best of our admittedly superior practitioners of the healing arts. For in it is the key to the standards and traditions of professional medical service.

Professional Medical Care

Informal aptitude tests must have been anciently applied to estimate ability and skill worthy to win popular approval for professional care of the sick. Native intelligence, pleasing personality, retentive memory, with accuracy in observation and skill in operation, and ability to apply knowledge effectively to the purpose at hand—these combinations of scientific and artistic endeavor must anciently, as now, have been the criteria for admission to the select group who were socially accepted as the healers and the nurses for the sick.

It is astonishing to discover these criteria in the oldest extant professional medical records, such as the Edwin Smith Surgical and the Ebers Medical Papyri of old Egypt. Copied about 1600 B.C. from older originals, these documents were medical students' texts. So were the more critical and sophisticated monographs of the Hippocratic school in Greek Cos in the 5th Century B.C. Standards of healing skill were even legally enforced in ancient Babylon, to judge from the provisions of the Code of Hammurabi of about 2200 B.C.

From these ancient origins the long and honorable tradition of western medicine has developed. For several millenia the emphasis in this tradition has been to treat sickness when it occurs. With every increment of verifiable knowledge about ourselves and our environment has come fitting application in the examination of the patient for the cause of the illness, in the organization of symptoms for convenience in classification in diagnosis, and for methods and principles of treatment, whether by drugs, surgery, or physical means.

To be clearly realized, however, is that the general tradition of professional medical service is to wait until sickness occurs, and then to do the best possible to alleviate symptoms or bring about a cure.

Various auxiliaries to the professional medical effort have been recognized from time to time. While pharmacy and dentistry are very ancient, and while nursing has a long record of sporadic service before Florence Nightingale, the notion of team-work for effective medical care is comparatively recent.

Even in the 18th Century, however, a distinctive hierarchy had developed, the effects of which unfortunately still linger. The developing American nation took over the traditions of 18th Century English medicine. In this the physician was usually a man of family with independent means, who had received a university training with a doctoral degree. He would condescend to give advice out of the kindness of his heart to those sick people who might be referred to him. He would not, however, send a bill. Accordingly, the custom was for the patient to leave a gold guinea on the hall table on departure. This is the basis for the internist's fee.

If the gentlemanly physician felt that an operation would be necessary, he would not deign to do it himself, but would refer the patient to a surgeon. The surgeon usually was a man without an independent income, and also without an M.D. degree. He was a hard worker, a practical man, and charged all the traffic would bear. The dentist was primarily a surgeon, whose special techniques developed in the 19th Century.

If drugs were indicated for the sick person, the gentlemanly physician would not deign to soil his hands by attempting to mix a recipe, but would send an order, a prescription, to the local pharmacist. The pharmacist was a merchant, certainly not of independent means, and not skilled in surgery. While his primary function was to compound the prescription sent to him by physicians, he also served as the first line of medical defense in the case of illness. Usually he was the first one consulted by a sick person, even as he is today. Since the 18th Century pharmacist's education was fairly good, thanks to guild regulations, his advice would be reasonably helpful, except in serious situations. Here it was expected that the pharmacist would refer the really sick person to the physician.

The general implications of this tradition are still with us, in spite of the early attempt in the United States to make all doctors capable both in medicine and surgery, as reflected in

the ordinary state license. With the rapid growth of special skill necessary for effective medical care, team work in giving such service is extending, and it is still appropriate that the physician call the signals. It is also wise for the physicians to remember that even though he may be calling the signals, he is still merely part of a team, all of which must function smoothly, if the patient is to get well.

The difficulty in this traditional form of medical care is that all members of the team sit back and wait for sick patients to show up. The emphasis is so strongly on fee for service, that no attempt has been made systematically to approach the problem from another angle. Ethics and economics become hopelessly confused in the traditional method of medical care. There is much hippocracy, both conscious and unconscious on the part of physicians, in professing the highest sort of moral principles, and then following with the callow business of charging and collecting a maximum fee for minimum service. The 18th Century gentlemanly English physician would never deign to make a house call. His 20th Century followers seem to be getting equally as gentlemanly. The pretensions involved in the hierarchy existing in the current mode of medical service tend to develop an elaborate etiquette. While the development of professional medical organizations has ordinarily the highest motives in promoting professional skill, standards, and attainment, inevitably the organizations tend to become political instruments. There is thus laid a trap to confuse purposes appropriate to a profession on the one hand and to a union on the other. It would seem wise for the professional medical organizations to maintain steadfastly their primary purposes (1) to promote professional ability; (2) to maintain the highest standards possible in professional education and training, and (3) to maintain the best achievement in professional conduct and activity. This means simply that state medical societies as well as national medical societies would be wise to devote their annual meetings and their publications to technical professional matters, leaving their political action discussions to informal sessions and to mimeographed bulletins for member distribution.

Prevention of Disease

Thanks to the great contributions of the scientists of the 19th Century, confirmable theories regarding the cause of infectious diseases were established. Here it is wise to remember the great individual achievement of Louis Pasteur (1822–1895), who was not a physician at all but a chemist. It was a great physician, however, William Henry Welch (1850–1934), who fully appreciated the significance of the new ideas on the etiology of disease. "Popsy" Welch deserves more credit than anyone else for the new concept of handling disease by preventing it.

The new idea of approaching medical problems by preventing disease involves two aspects: (1) individual immunization of people, which can be carried on by individual physicians, and (2) social control of sources of infection, by public health organization and management. With the expansion of the concept of preventing disease by including deficiency diseases, a broader program is capable of development under the initiative of individual physician.

Unfortunately, for the members of the medical team, the concept of prevention of disease is viewed chiefly from the standpoint of public health efforts, on the general basis of "let George do it." It is interesting that effective public health measures are slow to develop locally. They have been handled more successfully at state levels, and most effectively of all at national levels. The success of public health control in connection with the Armed Forces has well focused attention on the value of the national public health effort. Unfortunate, however, is the political repercussions of national public health effort, as a result of assumed jeopardy of individual incomes of physicians and other members of the medical team, who think they would lose money if public health service were to be extended.

There is always the danger that a large scale mass social activity such as public health, can become routine, dogmatic, authoritarian, and otherwise show evidence of stupidity and inefficiency. On the other hand, public health activity in assuring safe and sanitary water and food supplies, sewage and refuge disposal, epidemiological survey and control, and in many other aspects, can be wisely, helpfully, and sensibly developed.

Individual endeavor in the prevention of disease has not been as satisfactorily developed as might be the case were physicians and their associates a little more imaginative. The difficulty seems to be economic. The individual physician feels that any attempt to try to practice medicine on the basis of preventing disease would remove a major source of income. There is no logic in this position, and experience is against it. In whatever community a full-blown public health picture has developed, along with individual endeavor toward the prevention of disease, there has been an increased health awareness on the part of the people, with resulting increase in calls on the services of the health team. For many decades dentistry has thrived on the individual practice of prevention of mouth disease. Dentists have developed a superb method of practicing preventive medicine with satisfaction both to patients and themselves. In some communities, pediatricians and obstetricians have also successfully practiced preventive medicine, with individual arrangements with patients or families for the fees involved.

It would seem to be advantageous for physicians to attempt to develop a complete health service on the basis of prevention of disease. Preventive medicine could become the real specialty for general practitioners. An appropriate arrangement would be simply for each individual physician and his associate to make such agreements on an individual basis with families or persons, for regular health service, for a regular fee. This health service could include periodic examination, advice and discussion regarding mental affairs, nutrition, exercise, and general promotion of a healthy attitude toward living. The service could include immunization proceedings, advice with regard to prevention of deficiency and degenerative diseases and regular inspections for the early detection of diseased conditions.

The organization of health service for the effective practice of preventive medicine would involve all those who are concerned in caring for the sick. The usual clinic arrangement could be amplified and developed, to include regular follow-up and examination, as well as regular periodic supervision of diet and personal habits. With the expansion of effective methods for the practice of preventive medicine, team work could be promoted

more in accordance with the traditional service ideals of medicine. Doctors always profess the highest ideals with regard to rendering service to the people. Unfortunately, they and their families have to live. It is necessary therefore that doctors charge a fee. Up to the present the usual source of a doctor's income is fee for service when the patient is sick. It is incumbent upon physicians now to work out effective means for deriving their income honestly from aiding in the prevention of disease, rather than to wait for disease to come, and then to charge for treating it. If doctors were to derive their living from the prevention of disease they might really be interested in doing everything they could to stop disease and to promote the health of their patients.

Promotion of Health

The gradual transition and point of view in the health services from the treatment of disease to the prevention of disease, suggests a further logical extension. This is to take the emphasis off disease entirely, and to place it upon health. The program for the health services would then become the promotion of full optimum abundant health for everyone.

The success of the mental health effort in the United States suggests the opportunity for effective team work in the health services in the promotion of health. There are several factors involved. Motivation is required on an individual basis, effective techniques are to be learned, and enthusiasm maintained through group success.

Here is a challenging new idea which should excite the imagination of health educators everywhere. It is peculiarly appropriate to state supported health centers. The examples at state health centers might indeed be the triggers setting off extraordinary explosions of zeal for a worthy new proposal. In the Armed Forces current program, great success has already come from a deliberate attempt to promote optimum health and efficiency. It is no longer necessary for the health services in the Armed Forces to give chief attention to disease.

To promote an effective program designed to increase the joy and scope of living among our people generally will call for much goodwill, great forbearance, keen intelligence, and stimulating

leadership. If successful the results could be of tremendous significance for humanity. The promotion of intelligent morale among a people generally might indeed provide that security, that good health, that joy in living, which would produce the greatest group happiness.

Team Work in the Health Services

For the successful promotion of the best health possible for our people, extraordinary efforts will be necessary in the development of team work among experts in many diverse fields. Health includes not only good physical health and good mental outlook, but also economic stability and security, as well as clean, comfortable and convenient physical surroundings. The challenge to the best possible health for our people is therefore a call to the abundant good life.

From the practical standpoint of the health services, however, the matter can be narrowed to the proposition of promoting the best possible physical and mental health. An imposing program is already underway in the field of mental health. The techniques here developed might be fruitfully applied in other aspects of the health program. The example given by the Armed Forces Health Service might also be followed to advantage.

For half a century now, we have witnessed the extraordinary success of effective team work among the health services in the field of child health and welfare. The pediatric program has won a warm response from people everywhere, and our people are immeasurably the better for it. It has established the firm foundation for continuance into adult life, and indeed in preparation for old age. The program has involved research, organization, teaching, and then practical application along pragmatic lines toward the goal desired.

Health centers might well take this example seriously. Doctors are learning to work more effectively all the time with dentists, pharmacists, nurses, clinical laboratory technologists, radiology technologists, physical and occupational therapists, veterinarians, medical case workers, sociologists, clinical psychologists, public health officials and workers, hospital administrators, and even medical artists and librarians! Here is a remarkable list of health auxiliaries. These constitute the elements of the team involved in

the program for the best possible health for our people. Naturally the doctors should be the leaders.

It is incumbent upon the members of the medical profession to do everything possible to encourage the best sort of understanding among all members of the health field that they are working together toward common ends, and working together as a team. It might be appropriate to suggest that members of the medical profession will have to learn individually to give increasing respect and attention to all other members of the health team, in order to promote good team work, cheerful morale and effective results.

Medical organizations at the local, state, and national levels, might be wise to invite representatives of organizations comprised of other members of the health team to meet with them at regular sessions for the discussion of health team work. It might be appropriate to suggest the development of over-all conferences at local, state, and national levels, with representation from all of the organizations involved in the health team, for the purpose of developing good team play, and a practical program. Certainly John Q Public and his wife would be interested. Here would be afforded an extraordinary opportunity for the development of the democratic principle in relation to our most important asset, which is our individual and collective health. Such a proposition might be worth the careful consideration of some of the great new foundations which are being established for the promotion of our welfare.

At the World Medical Association Conference on Medical Education in London 1953, broad considerations are planned for selection of medical students, aims and content of medical curricula, technique and teaching methods in medical education, and the concept of social medicine and its place in the medical curriculum. Such conferences might profit from discussions of the proposed health team, including all the different health workers and the coordination needed in their training, so that their contributions to the health team may be integrated to the best possible advantage of the people they may serve. Emphasis on the promotion of health would aid in a satisfying approach to "social medicine."

The current great waste of premedical students, through failure of all but a small proportion to enter medical schools, might be averted by encouraging consideration in the pre-health curriculum of the future potentialities of professional training in the various auxiliary medical services. While actual training programs for the different health team workers would vary in accordance with the character of the service concerned, standards of admission similar to medical schools might be worthwhile in order to assure maturity of judgment and a helpful broad background for successful health team work. The concept of the health team, involving many differing health services might prevent much frustration among young people by offering opportunities for effective health team work even if they might not make the grade for admission to medical school. Challenging opportunities such as this require vivid imagination, much enthusiasm, strong determination, and generous goodwill. These are the ingrediences of success. Agreeable adaptations would be implied in connection with all who would serve, and wholesome appreciation of the contributions made by each group would be wise for all.

Health team work on a broad scale could well become the major objectives of state university health centers. Here could be brought together research workers, teachers, and expert service personnel to represent all parts of the health team. Working in common with close association with each other, so that recognition could come of the importance of all in the team, would engender mutual stimulus, inspiration, adaptation, and understanding. Perhaps under these circumstances the artificial, social, economic, and prestige barriers that now exist between the various health workers, could be eliminated.

Why should we not dream of "Utopias"? It is only by giving ourselves noble and exalting visions of what might be, that we are able to make even faltering steps toward them. The obligation is particularly heavy on members of the medical profession to show that generous understanding of the situation which would encourage the confidence of all other members of the health team. There would then come the necessity of justifying the confidence. There is no greater morale force than the compulsion

to justify a trust. Perhaps we can learn to take a few steps toward a health Utopia.

Summary

In considering the expanding tradition of health service one can trace four phases involving primitive methods of medical care by relatives, friends, and priests, organized and systematic care of the sick by professionally trained personnel, efforts toward the prevention of disease by public health service and by individual immunization, and finally in emerging programs for the development of the best possible physical and mental health of people. Many aspects of the old traditional approaches survived and will continue to remain as new logical expansions occur. Increasingly apparent is the general awareness of the value of team work in regard to the health services. Many specially trained persons are currently comprising the health team, and others may profitably be incorporated. As now constituted the health team includes doctors, nurses, dentists, public health workers, pharmacists, veterinarians, clinical laboratory technicians, radiology technicians, physical and occupational therapists, sociologists and case workers, clinical psychologists, hospital administrators, librarians, dieticians, illustrators, and medical and biological research workers. In each of these categories there is ample opportunity for a rich professional career. University health centers for reseach, training, and service in these fields have an inspiring opportunity for still further expanding the rich tradition of the health services.

TRAINING AND UTILIZATION OF TECHNICAL PERSONNEL

F. H. Arestad, M.D.*

Nearly twenty years ago the Council on Medical Education and Hospitals of the American Medical Association was asked to undertake the standardization of technical services ancillary to the field of medicine. This gave the medical profession a further opportunity to help develop adequately trained personnel for technical functions required in hospitals, medical schools, community health services, outpatient departments and clinics and the many doctors' offices which reach into the home areas of every section of the country. It was recognized, also, that these ancillary workers should be trained to work in co-operation with and under the guidance of medical personnel as essential adjuncts in the care and hospitalization of the sick. Thus each individual becomes a part of the health team that is necessary to serve the needs of community life.

In carrying out this program the Council has developed standards in medical technology, x-ray technique, physical therapy, occupational therapy and medical record library science, all in collaboration with the respective professional and technical organizations. These requirements are broad and give reasonable latitude and flexibility in the development and organization of educational programs. Emphasis has been placed on competent medical and technical supervision and the availability of adequate facilities and teaching material. Organizational methods are likewise described, faculty requirements, educational prerequisites, curriculum content and the length of training required in individual fields.

Medical Technology

The approval of schools of medical technology was initially a responsibility of the American Society of Clinical Pathologists and its Board of Registry. In 1933, however, the Society, realiz-

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ing the need for inspection, requested the Council on Medical Education and Hospitals to aid in the study and evaluation of laboratory training programs. After inspections had been completed and standards established in 1936, the Council published a list of 96 acceptable schools which have since increased to the present total of 496.

Under the Council's standards acceptable laboratory schools may be established in approved medical colleges, hospitals or state health laboratories affiliated with hospital services. The entrance requirements are two years of accredited college including 12 semester hours of biology, 6 semester hours of inorganic chemistry and 3 semester hours of quantitative analysis, organic chemistry or biochemistry. This is followed by at least 12 months of practical hospital training in biochemistry, hematology, bacteriology, parasitology, histology, serology, urinalysis, basal metabolism, and miscellaneous clinical microscopy. The approved laboratory schools may, if they so desire, establish affiliations with colleges and universities accredited by the respective regional associations. More than half of the present schools have availed themselves of this opportunity and are thus receiving a greater number of qualified applicants. On the other hand, the affiliated colleges and universities, by establishing adequate preprofessional curricula, have effectively broadened their educational service and are better able to channel their students into technical fields. Many of these programs lead to a bachelor of science degree in medical technology on completion of three years of college and one full year of hospital training.

The director of the school must be a graduate in medicine who is certified in clinical pathology or has had training and experience in this field acceptable to the Council. At least one salaried instructor is required who is a registered technologist or eligible for registration and has had a minimum of three years of experience. In laboratory practice the enrollment should not exceed two students to each member of the teaching staff. The hospital supplying the teaching material must be registered by the American Medical Association and have at least 2,000 yearly admissions.

The American Society of Clinical Pathologists, through its constituent Board of Schools of Medical Technology, is again co-

operating with the Council in the further inspection and evaluation of laboratory training programs. A preliminary survey has recently been made and will be followed by regular visits of inspection. When these studies have been completed the final decision as to approval or disapproval will, as in the past, rest with the Council on Medical Education and Hospitals.

Significant gains have already been made in medical technology as evidenced by the present enrollment of 3,500 compared with 700 in 1936. However, there is still a disturbing shortage of trained technical personnel as current estimates indicate the need for 10,000 additional workers to fill existing vacancies in hospitals and outside areas.

X-Ray Technique

The approval of schools of x-ray technique was originally carried out by the American Registry of X-Ray Technicians sponsored by the American College of Radiology and the American Society of X-Ray Technicians. This method continued until 1943 when the Council on Medical Education and Hospitals was asked to prepare standards, inspect courses of training and develop lists of acceptable x-ray schools. Complying with this request, the Council established educational standards the following year and in 1945 published a list of 112 approved schools which now total 310. The present requirements specify that acceptable schools may be conducted by approved medical colleges, general hospitals or x-ray departments affiliated with a general hospital. Preliminary college education is not required in this field as students may enter directly after four years of high school or graduation from an accredited school of nursing. While the minimum length of training is 12 months, 165 of the present schools offer longer assignments including 120 programs organized on a two year basis. The curriculum includes elementary anatomy and physiology, physics, x-ray equipment, dark room chemistry and procedures, x-ray techniques, ethics, record keeping and general office or departmental work.

The directing radiologist must be a duly licensed physician, certified or eligible for certification. At least one assistant instructor is required who is registered or eligible for registration,

with the further recommendation that one instructor should be available for each two students. The hospital used for teaching must be registered by the American Medical Association and have adequate clinical facilities.

The x-ray schools have recently been inspected by representatives of the American College of Radiology working in close collaboration with the Council on Medical Education and Hospitals. This survey has shown that the great majority of schools are operating in general conformity with the present standards, and in only a few instances was it necessary to recommend that the training program be completely reorganized. Continuation of the co-operative plan has been assured and the American College of Radiology is likewise participating in the evaluation of new applications. Inspections are made by qualified radiologists whose reports are reviewed by an advisory committee of the College and later forwarded to the Council on Medical Education and Hospitals for final consideration and action. Since 1945 the number of students has increased from 478 to 1,907, yet a shortage of approximately 5,000 trained x-ray technicians has been reported in relation to present needs.

Physical Therapy

The American Physical Therapy Association was responsible for the approval of schools from 1928 to 1936, after which the Council on Medical Education and Hospitals was asked to undertake the inspection and standardization of training programs in this field. After standards had been prepared in collaboration with the Council on Physical Medicine and Rehabilitation of the Am. Med. Assn., the American Congress of Physical Medicine and the American Physical Therapy Association, a list of 13 acceptable schools was published in 1936 on the basis of individual inspections. Under the present requirements of the Council acceptable schools may be established in medical colleges and hospitals approved by the American Medical Association, or in colleges and universities affiliated with acceptable hospitals and accredited by the respective regional associations. Students are eligible for admission who have graduated from an accredited school of nursing or physical education or have completed two

years of college with satisfactory courses in biological and physical sciences. The admission requirements have been increased in individual schools, however, and at present only 7 of the 31 approved schools admit two year applicants whereas the others require three or four years of college except in relation to graduates in nursing or physical education. It is apparent, therefore, that physical therapy training programs are now generally seeking a college level either through the establishment of a three year admission plan or the development of a regular four year degree course as now maintained in 11 schools.

The school must operate under the direction of a physiatrist or other physician whose qualifications are acceptable to the Council. The technical director should be a qualified physical therapist with 2 and preferably 3 years of general experience, training in educational methods and tests, should be registered or eligible for registration, and should possess an academic degree. Where clinical practice is carried out there should be at least one qualified therapist for each six students. The hospital serving the school must be approved by the American Medical Association and have adequate clinical material for teaching purposes.

The minimum length of training in physical therapy is 36 weeks with approximately one-third devoted to didactic instruction and two-thirds to practical assignments, demonstrations and clinical practice. The program is organized in relation to medicine, surgery, neurology and orthopedics and involves also the applied sciences of anatomy, pathology, physiology, psychology and physics and specific procedures in electrotherapy, radiation techniques, hydrotherapy, massage and therapeutic exercise. It has been estimated that 5,200 physical therapists are currently employed with 80% in hospital service. It is likewise the opinion that at least 4,000 additional trained workers are required to fill existing vacancies in hospitals and outside areas. The approved schools, therefore, with only 600 graduates a year, are not able to meet the demands for well qualified technical personnel in this field.

Occupational Therapy

In 1933 the Council on Medical Education and Hospitals was asked to establish educational standards in occupational

therapy and undertake the evaluation and approval of individual schools. An inspection program was instituted shortly thereafter and standards were developed in co-operation with the Council on Physical Medicine and Rehabilitation and the American Occupational Therapy Association. In these requirements it is specified that occupational therapy schools should be established only in medical schools approved by the American Medical Association or in colleges and universities affiliated with acceptable hospitals and accredited by the respective regional associations of colleges and secondary schools.

The director of the school should be a qualified occupational therapist with at least 3 years of clinical experience, who has an academic degree and is registered or eligible for registration. The clinical training, however, should be under the direction of a physician or committee of physicians whose qualifications are acceptable to the Council.

The minimum length of training is 100 weeks including 39 of theoretical instruction, 25 of technical training and 36 of hospital practice. The theoretical instruction includes 18 semester hours of anatomy, kinesiology, neuroanatomy, physiology and psychology, 4 semester hours of social sciences, 8 semester hours devoted to the theory of occupational therapy, 7 semester hours in clinical subjects and 2 semester hours of electives. Flexibility in technical requirements is emphasized but training equivalent to 25 semester hours should be provided in the major fields of (1) arts and crafts, (2) education, and (3) recreation. Then follows a period of 36 weeks of clinical training with assignments in hospitals or other clinical centers covering at least 12 weeks in psychiatry, 8 weeks in physical disabilities, 4–8 weeks in tuberculosis, 4–8 weeks in pediatrics, and 4–8 weeks in general medicine and surgery.

The educational plan in occupational therapy follows the college pattern. Nearly all of the approved schools admit high school graduates to courses that are mainly of 5 years' duration. In addition, certificate programs are offered for college graduates who in practically all instances are given 18 months of occupational therapy training. A few schools also admit two year applicants for 27 months or one year college students for a minimum

training of 4 years. It follows, therefore, that educational programs in occupational therapy are seldom completed until the student has been out of high school at least 5 years. This long period of training is now regarded in many areas as a significant factor in the continuing shortage of qualified personnel. At present there are approximately 3,800 registered occupational therapists with an estimated requirement of 4,500 additional workers to fill existing vacancies. The need has accordingly reached a critical stage, especially since the 25 schools currently approved by the Council report only 500 graduates a year.

Medical Record Librarians

In 1942 the Council on Medical Education and Hospitals was asked to assume responsibility for the approval of schools for medical record librarians. The following year a set of standards was developed in co-operation with the American Association of Medical Record Librarians and a list of 10 acceptable schools was published on the basis of individual surveys. The requirements of the Council provide that acceptable training programs may be established in approved general hospitals that have a minimum of 6,000 annual admissions, or in colleges, universities and medical schools having suitable hospital affiliations.

The medical record librarian in charge should be registered or eligible for registration and should have (1) two years of accredited college, graduation from an approved school for medical record librarians, and three years' experience in charge of a record department in an approved hospital, or (2) other equivalent educational qualifications and experience acceptable to the Council.

Students are eligible for admission who have completed two years of accredited college or have graduated from an approved school of nursing education. The length of training is at least 50 weeks in which 270 clock hours are devoted to theoretical instruction including 100 hours in anatomy and physiology, 30 in clinical subjects, 30 in medical terminology, 100 in medical record procedures, 10 in departmental management, and (25) in psychology if this subject has not been suitably covered in preliminary courses. The remainder of the year is devoted to

practical training with assignments covering approximately one week of orientation, 8 weeks in the admitting section, 10 weeks in discharge procedures, 10 weeks of indexing and coding, 8 weeks of surgical dictation, comparative and group studies and 5 weeks in record activities of adjunt departments.

In this field the shortage of trained personnel is particularly acute. The number of registered librarians totals approximately 2,600 and it has been estimated that 4,500 additional qualified workers are needed to fill existing vacancies. As the 21 schools currently approved by the Council supply less than 100 graduates a year it has been proposed that consideration be given to the establishment of additional training programs for students directly out of high school.

Additional Needs

While great progress has been made it has not been possible to meet all of the needs for well trained technical personnel. In this continuing shortage many factors are no doubt involved such as the lack of suitable educational facilities in many areas, the increasing competition with other fields, the mounting cost of education, expanding entrance requirements, the inability of high school students to enter directly into technical training except in x-ray technique, high attrition rates, limited facilities for male students, inadequate economic returns, insufficient publicity or recruitment activities, incomplete development and integration of preprofessional courses, longer periods of training and the growing apprehension that in some fields the existing standards are already exceeding the bounds of practicability.

The medical profession and the respective technical organizations are cognizant of these needs and are making every effort to promote the training of ancillary personnel. In this connection, the Council on Medical Education and Hospitals has been in communication with all medical schools calling attention to the need for further facilities for the training of physical therapists and occupational therapists. From the replies received and subsequent studies and contacts it is apparent that many additional programs will be developed in these fields. Where educational services are already in operation the medical schools

recognize the existing shortages and are prepared to maintain enrollments at the highest level consistent with good teaching.

Promotional material has been widely circulated by each of the technical groups but limited resources have hampered the development of extensive recruitment. However, the Health Information Foundation is now giving serious attention to the lack of well trained health personnel and is attempting to correlate the recruitment activities of the technical organizations and enlist the aid of the Advertising Council which for many years has given such generous and effective support to the nurse recruitment program. It is hoped that this undertaking will be successful in bringing large numbers of additional applicants into the technical fields.

Colleges and universities can likewise give valuable aid not only in the counseling of individual students but also in the development of further preprofessional courses that may lead to subsequent technical training. Whenever possible these programs should be closely integrated with approved technical schools to develop continuity in training and more effective teaching at both levels. Often it is advantageous that affiliations be entered into not only in relation to degree courses but also when one or two year college students are accepted for hospital assignments in accordance with minimum requirements in individual fields. These closer liaisons will likewise create a better understanding of educational problems and may, through the interchange of information, give valuable help in the formulation of policies or the development of new methods or patterns of education.

A further evaluation of the existing standards in physical therapy, occupational therapy and medical record library science is now under consideration with particular reference to length of training, entrance requirements and curriculum content. Already there appears to be general agreement that 5 year courses should be reduced and that degree programs in the technical fields should be kept within the framework of a regular four year plan with appropriate credit assigned to the required period of hospital training. It has also been proposed that basic courses of shorter duration be established in each field and that hospital schools be developed in occupational therapy to sup-

plement the collegiate program. Many of the problems associated with the supply and production of competent technical personnel are likewise related to the academic fields where the initial preparation is made and where counseling and recruitment may serve to direct students into areas ancillary to medicine. Every effort should, therefore, be made in colleges and universities to create additional opportunities for technical training in close affiliation with hospitals or other medical centers. While there is continuing need for four year degree courses to supply directing and supervisory personnel, attention should also be given to the development of shorter programs that can provide the bulk of technical service now required. Perhaps a two year certificate program, well organized and closely integrated with the hospital field, should be considered as a means of accelerating the production of essential personnel. In this connection, it is urged that closer liaisons be established so that all who are concerned in the training and utilization of technical personnel will be better able to determine their respective areas of responsibility in the overall planning of preprofessional and technical education.

THE COLLEGE CURRICULUM AND DENTAL EDUCATION*

SUMTER S. ARNIM

No single profession has profited more during the past two decades by advances in college educational technics and methods than the dental profession. We are grateful to educators in our colleges and universities for providing the highest type professional students it has been our privilege to teach. This improvement in the quality of students attending dental school has not been brought about by specific efforts such as the one we are now engaged in. It has grown primarily from the work of college and university educators.

I am a product of the school system of the State of Texas. As I remember my days in college, pre-medical education was a new idea. No one at that time had definite convictions about preprofessional requirements, nor were we blessed with the many examining councils and boards that, at the present time, are establishing rigid requirements for certain professional activities. Most medical and dental schools were glad to enroll students, preferably male, and it was considered desirable that the entrant could read and write. College and university educators were performing a marvelous job during those days.

Johns Hopkins was one of the first professional schools to emphasize the desirability of a college education as preparation for a profession. There has been a steady improvement in the university and college preprofessional training, quantitatively and qualitatively. Certainly we can hope through conferences of this sort to build on the background of experience and continue to provide a better health personnel and service for our citizens.

In order that we all have a clear idea of the aims and objectives of dental education, so that we may discuss more intelligently the relation of the college curriculum to dental education, I shall quote from *A Course of Study in Dentistry*, which is the report of the Curriculum Survey Committee of the Ameri-

*From The University of Texas Dental Branch, Houston. Presented at the Preprofessional Health Conference held at The University of Texas Medical Branch, Galveston, May 15–17, 1952.

can Association of Dental Schools. This report was made in 1935. Many of the thoughts expressed are still useful for present-day thinking. In this report, the objectives of undergraduate dental education are defined in these terms:

Dental education "should aim at training students in order that they may

A. Be competent in the maintenance of oral health and the treatment of oral diseases, disorders, and deficiencies, with understanding and appreciation of the relationships between oral and systemic conditions in health and disease.

B. Co-operate effectively with persons engaged in allied fields of service.

C. Have interest in and desire for continuing professional study after graduation.

D. Practice dentistry with due regard for its social, economic, and ethical relationships.

E. Co-operate effectively in community life."

In addition, the following statement is made: The dentist should possess "certain essential qualities, among which are honesty, tact, moral integrity, and ability to deal with people."

Examination of these objectives and aims of undergraduate dental education reveal to us the larger part of the dentist, as a person, comes from the educational effort of others than dental teachers. Aim "A", to be competent in the maintenance of mouth health and in the treatment of mouth disease, is a specific responsibility of the educator in the field of dentistry. To be able to "co-operate effectively with persons engaged in allied fields of service, to have interest in continuing professional study, and to practice dentistry with due regard for its social, economic, and ethical relationships," as well as to "co-operate effectively in community life," are all attributes that grow with the child through school, college, university and professional school. Certainly, parents, educators, and dental teachers contribute a bit here and a bit there to this development of the individual as a whole. This is as it should be.

The objectives just outlined were formulated in 1935. In recent discussions at The University of Texas, Dental Branch, concerning educational aims of our institution, the faculty recognized its responsibility to provide for the citizens of Texas,

dentists who are prepared to accept their whole responsibility, both as well-informed citizens and as competent dentists. To attain these qualifications in the dental graduate, the faculty recognized that certain disciplines need be taught without regard of course content. These disciplines common to all courses should build qualities such as need for religion, a sympathetic understanding, honesty, integrity, a quiet manner, the desire to be of service to others, the ability to listen, the technic of understanding, the desire to get along with people, and how to be wholesome.

During these discussions it was brought out that the sciences, both clinical and basic, should be taught in a manner that the needs of the majority of patients, who visit the average dental office in the average community, can be supplied by the dental graduate. The study of the whole patient, as well as the entire mouth, is essential for complete diagnostic service and treatment. Understanding of the dentists's own limitation in clinical practice should be taught as reason for learning in the area of consultation with emphasis on the "when to consult" and the "when to refer." Comprehension of these principles will lead to return of the dentist for advanced education when he desires to supply specialized services himself. The faculty also accepted as their responsibility the teaching of certain business technics in order for the dentist to meet community and office needs. It is necessary that he have an understanding of the principles and technics of business as they apply to the practice of dentistry.

The college teacher, while contemplating these aims and objectives, both old and new, can readily see many applications for his special field of interest in a pre-dental course. Experience has shown that college training should include, quantitatively speaking, certain required courses in order to provide the student with the necessary "know-how" at the time he reaches dental school. These disciplines are listed in the catalogue of the Dental Branch (2) in these words: "The scientific discipline should include mathematics, chemistry, physics, and biology. The discipline in the humanities should include English composition and literature, a foreign language, history, philosophy, and other social sciences." These pre-dental requirements of 1952 have replaced the high school diploma for prospective dental students

of 1910. In fact, it is now considered preferable to be a college graduate, male or female, prior to entering dental school. The need for excellent preparation in the sciences, both quantitatively and qualitatively, is well-known and understood by all educators. The student with poor preparation in science is soon so far behind his better trained fellows that further progress in professional education is impossible, and he is lost to the cause. The Dental Branch provides a catalogue and special information sheets from time to time, which are mailed at request of those interested, concerning requirements for admission. Requirements change from time to time.

Perhaps some of you wonder why so little is said about the needed and necessary training of the dentists' hands. The practice of dentistry is the practical application of skilled co-ordination of mind, eye, and hand for treatment of diseases of the mouth and teeth. Experiences useful for creating such skill in the student do prove helpful in dental study and practice. Consequently, another requirement has been introduced recently for admission to dental school. It is a National Dental Aptitude Test given under the auspices of the American Dental Association. This test attempts to measure abilities related to the co-ordination of mind, eye, and hand, as well as give some idea concerning a student's preporation in the sciences and the humanities. The Dental Branch in Houston is one of the centers for administering the aptitude test, and it is a requirement for admission to the undergraduate dental school. It can be truthfully said that "handiness" or an aptitude with one's fingers is an attribute or quality much to be desired in the dentist. Students who have such dexterity developed to a high degree do better in dental school than those who are clumsy. Thomas Weeks (3) once wrote, "All practices at the college level that exercise the mind, the eyes, and increase the dexterity of the hand will contribute toward the preparation of a student for the study of dentistry. The ability to understand is also improved by seeing and handling and doing; in fact, systematic training with the hands is not antagonistic to mental growth, but may actually assist it." Need more be added concerning digital skills?

Today in most discussions of the relation of college curriculum to dental education, we find such a predominance of thought

concerning the building of a professional man, rather than emphasis upon details of the course content. The situation calls to mind a letter recently received from one of the dental educators of Sweden. Dr. Seipel, Professor at Malmo, writes, "The sectioning of a school in too many departments and subdivisions makes a fractional distillation of the student's mind and delays his development into a one-mind, one-man practitioner, which is the objective of dental education."

Dental education at its very best must be highly technical and largely devoted to the acquisition of special skills and technics. Consequently, in closing, may I leave this thought with you: at the college level, one cannot go wrong in stressing the humanities. Numbers, facts, hours and even courses are servants only. Teach for the major objectives of life, integration and co-ordination, development of judgment. Teach for life and for living, the building of a good citizen. Teach that all practitioners of health services love their fellow man. Teach that all doctors are their brother's keeper. Send us these men, so taught, and we will return to you a better dentist for tomorrow.

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NURSING EDUCATION IN THE HEALTH CRISIS

ELIZABETH S. BIXLER*

"Nurses are the most numerous members of the health team in this country. The availability of nursing personnel of all types in quantity and quality sufficient to meet national needs is an important factor in the health of our citizens. To the educational system of the nation falls responsibility for educating this large group of essential health personnel."—Lucile Petry and Ellwynne Vreeland, *Higher Education*, April. 1952.

It is less than 100 years since the Act establishing Land-Grant colleges was put into effect. In the history of higher education in the United States this action gave assurance that a college education should be available to the many, rather than to the chosen few who had heretofore enjoyed this privilege, and that a practical education could be obtained at the college level. Since that time we have seen the growth of universities and the affiliation of professional schools with the universities—schools of law, medicine, education, journalism, business administration and, more recently, schools of nursing.

Shortly after the Land Grant Act was passed schools of nursing were established in the United States as "training programs" in hospitals. The admission requirements to these early schools were such that the students, although not college women (since very few women were going to college in those days) were women of maturity (at least 21 years of age), cultured and well educated. By the time of the First World War, the importance of a college education for nurses was emerging. The Vassar Training Camp was established for college graduates to encourage this group to enter nursing and help fill the ranks of much needed Army nurses. Those nurses who had prepared at the Vassar Training Camp soon showed in their professional practice the advantages of a college education, and many of these same individuals are leaders in nursing today. Thus the two factors—the growth of universities and the growing importance of well educated nurses—converged, and we have the beginnings of the university or collegiate school of nursing.

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The first university program leading to a degree in nursing was set up over thirty years ago. The belief that nursing education should be established in institutions of higher education had a slow growth at first. This development has been more rapid recently, but even today there are only approximately 180 such programs, in contrast to some 900 schools of nursing offering a diploma program and administered by hospitals.

About twenty years ago the Association of Collegiate Schools of Nursing was established to foster the growth of college or university controlled programs in nursing,—programs which lead to a baccalaureate or higher academic degree. At present forty-nine of these 180 programs have membership in the Association of Collegiate Schools of Nursing, which means that they have met certain criteria set up by the Association. These criteria are concerned with the organization of the school, its financial support, the competency of its faculty and its selection of students. Specifically the standards for membership state, in part:

"The organization of the school shall be in accord with the general plan in effect in the university or college in which the school is located. . . .

"The curricula shall meet the requirements of the college or university for a baccalaureate or higher degree.

"All students must be matriculated for the degree, and the degree requirements must be met before the certificate of completion is granted.

"The college or university of which the school is a part must assume responsibility for the clinical experience (practice) and instruction.

"There shall be a definitely assigned budget sufficient in amount to maintain the school on a level commensurate with that of other professional schools in the college or university.

"The faculty shall have academic status and authority in accord with the policy governing faculty organization in the college or university of which the school is a part."

Approved collegiate programs in nursing leading to a degree follow two general patterns: One in which general education is integrated with university controlled professional education throughout the entire program. This is usually a four or five year plan. The other, in which courses in general education serve as a foundation for university controlled professional courses. This plan usually consists of two years in an approved junior or senior college, followed by three years, approximately,

of professional nursing education, including the clinical experiences in hospitals and other health agencies. There are arguments in favor of both. "Integration" is accepted as desirable, but since integration really takes place within the individual student, it may be provided for in either of the two approved patterns. The variations in nursing education today are naturally confusing. Rather than apologize for the absence of a rigid pattern, suffice it to say that nursing is an emerging profession, suffering the trauma of growing up.

Hospitals are an essential part of nursing education because one learns by doing, and one of the strengths of nursing education is that students learn by taking care of "real" patients. Research and experimentation are being carried on to determine possible improvements in the hospital-controlled or diploma program.

Other professions have discovered the value of the technician, less highly trained but equally important with the professionally prepared person. This pattern has been shaping up in nursing for the past several years, and is particularly significant now because of the terrific demand for nursing care. Highly skilled, professional nurses with a sound education are needed as teachers, as supervisors and at the bedside. But their services can be supplemented by those of the person with certain skills demanding somewhat less breadth and depth of preparation. There is, however, a firm belief among nurse educators, that nursing education, in order to give its students the best preparation for the service demanded of them, should be established in institutions of higher learning.

Everyone today is aware of the increasing demands for nursing services and with the fact that the supply has not nearly kept up with the demand. Patients, nurses, doctors, citizen and professional groups on local, state and national levels are all concerned, and all are trying to find the answers to the real problems presented by this situation. For some, the answer seems to be to give young people as brief as possible a period of training in order to swell the ranks of bedside nurses. Without ignoring the need for technicians, practical nurses, and the like, my special plea is for well prepared nurses, educated in our colleges and

universities, and capable of accepting the special responsibilities demanded of them by the profession and by society.

The National Committee for the Improvement of Nursing Services is one group that is seeking for a reasonable solution. This committee has recently prepared a chart which shows graphically the distribution throughout the country of professional and non-professional nursing personnel—in homes, hospitals, doctors' offices, industry and public health. It also shows clearly the proportion of professional nurses who are responsible not only for the care of patients, but for the direct guidance of other nurses and non-nursing personnel.

For example,—in the hospitals there are 128,000 general duty nurses, about 50,000 practical nurses and over 200,000 auxiliary workers. The general duty nurses are caring for patients, and supervising the work of the non-professional workers and the student nurses, as the latter groups are also essential in patient care. In hospitals, in schools of nursing and in public health there are approximately 72,000 instructors and supervisory and administrative personnel. Many of these individuals are assisting with the actual bedside care of the patient, but their main functions are to guide the work of others, and to teach the groups of oncoming students. It seems self-evident that such responsibilities call for the best possible education, and therefore that at least a third of the total nurse group, which includes the classifications I have listed, should have a college education.

Isabel Stewart, formerly Director of the Department of Nursing Education, Teachers College, Columbia University, has written:

"One of the questions that comes up frequently in our collegiate school conferences is the place of the college-trained nurse in the practice of nursing. Many seem to assume that colleges will train only the nursing leaders—those who are to teach and direct others, not those who actually care for the sick in homes and hospitals and 'carry the bag' as staff nurses in public health agencies. I am sure that this is not the conception most of us have of a professionally qualified nurse. We do recognize the need for more nurses who have the personal qualifications and educational background for leadership positions, but we know that they are not going to be fitted for such work unless they have first mastered the science and art of nursing and know the problems of nursing practice at first hand. The main difference between the broadly prepared professional nurse and the

nurse technician is that the professional understands better what she is doing and is more capable of relating herself and her professional work to the total social and cultural pattern. She is also better prepared to investigate and solve nursing problems and to plan for the future because she has had better training in thinking and more knowledge with which to think."

If we agree, then, that nurses are to have some college education, what are the pre-professional requirements? The bulletins of collegiate schools of nursing list certain courses as requirements for admission. In high school the student must have English, Mathematics, Natural Science. In college, English, Biology, Chemistry, Psychology, Sociology and History. Not a startlingly original list—as a matter of fact one that might be considered almost a "routine" preparation for adult life. Actually, "pre-professional requirements" are not met by any list of courses as such. If the aim of a liberal education is to prepare the young man or woman for life, over and above preparing him for making a living, it is the concepts behind the facts that are the important element in his college experience. Eldon Johnson of the University of Oregon, spoke on this subject at a meeting of the National League of Nursing Education a year or two ago. He pointed out that the special task of the liberal arts college is "to see man and life whole" . . . "to train a student not only as a doctor, lawyer, nurse, chemist, or engineer, but as a man and as a unit of society." "My plea" he states, "is that when young men or women go to our schools of education to become teachers, they have something to teach; when they go to our schools of nursing, they understand more about life than anatomy." (Eldon L. Johnson, 53d Annual Report, N.L.N.E.)

And why should a nurse know more about life than about anatomy? It may be helpful to take a look at the nurse of today to determine how she uses the knoweldge acquired in her pre-professional, as well as in her professional education. Everyone knows that nurses care for sick people. The key words are "care for" and "people." (Without belaboring the point it is evident that unless one does really care about people and what happens to them, one does not go into nursing.)

One responsibility of the nurse is to carry out the treatments as prescribed by the doctor. Because she is technically skilled

she can attend to the patient's personal hygiene and physical needs; she administers medications; she sees that he gets the proper amount and kind of nourishment; she adjusts the oxygen tent; she watches the flow of intravenous fluids; she notes the patient's physical symptoms, his temperature, pulse, respiration, blood pressure, color. Technical skills, yes, but demanding in addition to deft, trained hands, a basic knowledge of anatomy, physiology, physics, chemistry, disease processes, and both the salutory and untoward reactions of many different drugs; demanding also keen powers of observation to denote the subtle changes in the patient's condition which may be danger signals.

Uppermost in the mind of the nurse, in these aspects of nursing care, is the *comfort* of the patient. Here her technical skills merge into the art of nursing. A smoothly made bed and a good back rub give the patient greater physical comfort, but while she is carrying out such routine measures, the nurse bears in mind the patient's mental comfort as well. She is listening, answering questions, observing—in other words she is caring for a *person*, not for an appendix or a cardiac care. The patient, because he is sick, has specific needs which go beyond medications, treatments and diet. It is a further responsibility of the nurse to recognize these needs,—the need for attention and recognition, for security and reassurance. It is a well accepted fact that one's emotions play a large part in one's reactions to illness. Fears, anxieties, tensions, must be allayed. The nursing care of such symptoms varies with the situation. It may consist of calling for the doctor and his professional skills, or for the social worker to straighten out a home situation, or on the clergy for spiritual help to the patient; or the patient's mind may be eased by the words of the nurse, or by her attitude alone. An intellectual and emotional grasp of the fundamentals which govern the behavior of man is essential to the art of nursing. And because the patient is a distinct entity formed by his own particular hereditary and environmental influences, he has to be treated and cared for as an individual person, different from every other individual.

The Russell Sage Foundation has recently sponsored an interesting piece of research at the New York Hospital. The report has not yet been published, but should be well worth reading by professional health workers and by educators interested in

pre-professional preparation. Professor Leo Simmons of the Department of Sociology of Yale was assigned by the Foundation to the New York Hospital to study the sociological aspect of the role of the nurse. In the course of his two-year study, he became well acquainted with the hospital situation, by day and by night; he talked to patients, to doctors, to nurses. In a paper entitled "The Manipulation of Human Resources in Nursing Care," which he read a year ago in Boston, he makes the following comments:

> "But there is another level of reactions of equal importance, and, for short, we have called it the level of interpersonal relationships. There is the sick man, a *person* as well as a body. There are powers of personality within him for healing and for health, and also for sickness and death. Associated with him are other persons equally endowed with such powers. We know, and it can be demonstrated experimentally, that the relationship of these persons to one another and to the patient are laden with both constructive and destructive possibilities. Every physician and nurse knows the difference it can make in a sick person to feel rejected and without interest in the fight for life, on the one hand, or warmly wanted and stimulated to live, on the other. The balances here are often pivoted on a fine point, and it is not infrequently the little human touches that tip the scales. A head nurse said of a depressed patient recently—'If only we can get to him, I think we can save him.'" (Leo Simmons, 57th annual report, N.L.N.E.)

A nurse in the pediatric ward may be caring for a very sick child who has had cardiac surgery. In our hospital the directions for nursing care of these "Blue Babies" take up two mimeographed pages of instructions. Because these children are usually very dependent on their families and especially fearful at being brought to the hospital, the nurse must have insight and judgment to make the child's first adjustment as easy as possible. There are special procedures, calling for professional skills, in the pre-operative care; when the patient returns from the operating room, and is placed in an oxygen tent, it is extremely important that he be watched carefully for signs of the many possible complications. As our procedure states: "It is best that the same nurse be with the child continuously for eight hours as it is upsetting to a small child to have a different nurse answer his every call as he awakes from his fitful sleep." The nurse must be keenly observant and quick to act as the need arises.

Or she may be caring for a group of children who have been admitted to the hospital to have their tonsils removed the following day. These children are not "ill"—they are not put to bed until the usual evening hour. Some are frankly terrified and need comfort and reassurance from the warm, sympathetic nurse, who must serve as a mother substitute for the time being. Others are independent and are not going to show anyone that they are scared. But they are in a strange environment and separated from their parents. The nurse suggests that they play games after supper and she supplies the requisite toys. Soon the small wagon is an ambulance and the dolls are sick. The children are acting out their underlying fears. The nurse enters into the spirit of the play, and supplies some green masks and caps that are worn by the surgeon in the operating room. She lets the children try them on and explains that this is what they will see the following day when they go upstairs. By bedtime the children have played through their normal anxieties and with the reassurance that they will see the same nurse when they return from the operating room they go calmly to sleep.

The nurse in the maternity ward, particularly in the Rooming-In set-up, where the baby's bassinet is next to the mother's bed, is giving nursing care, in its wider connotation, to the family unit—mother, father, baby. Here I believe her attitude is almost as important as her words. She takes time to help the young father learn how to hold the baby. She appreciates his need to get acquainted with the new member of his family. She casually bubbles a baby against her shoulder while chatting with a mother or answering her questions. Questions are to be expected from young, anxious parents and the answers are relatively easy for the nurse because of her professional education; but the relaxed, optimistic atmosphere of the ward is a part of the art of nursing.

When she is in the public health field, the nurse is again caring for whole families. A large part of her work, as the term public health indicates, consists of teaching the principles of optimum health. She is active in well baby conferences, and in classes for expectant mothers. She may be assigned to the school system and be responsible for periodic health examinations and for teaching the rules of hygiene. She also has bedside nursing duties. She is called to a home because of a sick child. She gives

the necessary care and teaches the mother what to do for the child between the visits of the nurse. In the same home she may find another member of the family who to her trained eye, but unbeknown to himself, needs some specialized medical treatment. The situation calls for discernment, careful handling, knowledge of the social agencies of the community. Dr. Charles T. Bingham of Hartford, Connecticut, speaking on the subject "The Psychosomatic Approach to Nursing," has said:

> "If disease is produced by both physical and emotional stresses and strains, the person who comes into contact with the members of a young family early in life can often do more than anyone else to start them down the road towards happiness and health. On the other hand, such a visiting nurse inadequately trained in the dual aspects of disease may do endless harm in her sincere efforts to uncover unsuspected tuberculosis or early cancer. Cardiac cripples and hypochondriacs are often produced by thoughtless words of fear while endless healing comes from simple words of cheer." (*Nursing News*, March, 1950.)

To summarize, whether in the hospital or in the home, there are certain aspects of the work of the nurse which may not be immediately obvious to the public. ***First,*** she is carrying a heavy responsibility for the safety of the patients. The doctor sees the hospitalized patient for only a small fraction of the 24-hour span, but a nurse is always present, aware of changes in the patient's condition, alert to danger signals and quick to respond to them. *Second,*—she is continuously adjusting herself, her attitudes and behavior to the varying needs of the different patients. Calmly sympathetic with one patient's complaints, gay and joking with the next; solicitously attending to the needs of the elderly, bedridden, "incurable" patient, and the next moment encouraging another patient to assist himself toward rehabilitation,—no one needs adaptive powers more than the nurse.

Third,—the nurse is a member of a team. She works with the doctor, the social worker, the special therapists, carrying out her part of the overall plan for the care of the patient. She is also the team leader of the nursing group, guiding the work of student nurses, attendants and aides. And *fourth,*—the nurse is teaching,—teaching mothers to care for their new babies, teaching good hygiene, explaining symptoms, giving advice on self care to be followed at home when the patient leaves the hospital;

in other words, teaching positive health and how to maintain it at the optimum level. "She must understand," says Annie W. Goodrich, "both the language of science and the language of the people, because she is continuously interpreting the voice of science to the people in terms which are clear to them."

I hope I have made it clear why the prospective nurse must have a sound educational background in the physical and social sciences, in the humanities and in communication skills. I have tried to give a glimpse of the bedside nurse of today, in order to put some life into the list of required courses. There is time for only a brief mention of other fields in which nurses are active. The armed forces need nurses who have not only specialized professional skills but the requisite stamina to adjust to combat conditions, and to give of themselves beyond the call of duty. Nurses are employed by the World Health Organization and in the Point IV program, helping in rehabilitation programs for the underdeveloped countries. Nurses are faculty members in our universities, deans of schools of nursing, directors of hospital nursing services and of public health nursing agencies. They assist in planning public health proprams for small and large communities and regions. In administrative posts they frequently must be able to draw up and administer a budget of large proportions.

I recently heard Mr. Oliver Carmichael of the Carnegie Foundation point out that although only 20% of our 18-year-olds actually attend college, the university-trained individuals are the teachers in our secondary schools, the doctors and lawyers in our communities, and thus "directly or indirectly, higher education ministers to the entire population." That nurses working in the hospitals and in the public health field are important members of the community is an undisputed fact. With others on the health team, they are caring for the sick, teaching positive health measures to individuals and to groups, taking part in community health surveys for the prevention of illness. Thus they are also influential members of the community and as such should have the benefits associated with a university education.

A quotation which seems to me applicable, but the source of which I regret to say I do not know, states that:

> "The best educated person is one who has the tools with which to meet most effectively and satisfactorily the greatest number of situations in life. Education proceeds as experience is gained and is not concerned entirely with the accumulation of knowledge of facts, but is rather concerned with the way a knowledge of facts can be applied to daily life."

Nursing education today is finding its place in our universities. Higher education, as stated earlier in this paper, has a responsibility for both the pre-professional and professional preparation of a group of workers who have so much to contribute to the health of the nation.

THE ROLE OF A NATIONAL PREMEDICAL HONOR SOCIETY

H. E. SETTERFIELD*

The National Premedical Honor Society embarked on an educational program at a national level about eight years ago to meet the crying need for serious study and discussion of many problems relating to the preparation for the study and practice of medicine. But throughout these years the Society has not tried to propagate any special program or philosophy of education in distinction to any other. Rather the Society has felt that its value could be greatest if it could be the "neutral territory," so to speak, on which medical educators and premedical teachers and advisors could meet and discuss problems freely without the necessity of either one playing the host or guest. In the years since 1944 the Society has arranged three symposia in co-operation with the American Association for the Advancement of Science. For these programs speakers were obtained from medical and collegiate education of national note. One such program was arranged with the co-operation of the American Chemical Society.

All of the papers presented at these meetings have been published. In 1947 the Society organized the first of four Regional Conferences on Premedical Education. In that and in succeeding Regional Conferences, the involved area included about 100 colleges and universities. Delegates were invited from these colleges and universities and the medical schools in the same area to meet for two days of discussion of mutual problems. These four conferences have covered some 18 states, 300 undergraduate colleges and universities and 25 medical schools. Transcripts of the discussions as well as the formal papers presented have been published. In 1950 the Society arranged a two-day program in co-operation with the Association of American Medical Colleges. It was called the First National Conference on Premedical Education. Fifty-four medical schools and about

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200 colleges of the nation were represented at that meeting. A number of persons here today will remember that as a highly stimulating affair.

Through these years the Society has published not only the reports of all these meetings but many other papers dealing with various aspects of preparation for the study of medicine and the allied health fields. Not the least of these other publications was the report of the Survey of Premedical Education which was conducted by questionnaire in 1945–46. This Survey did much to stimulate thinking among medical educators about the problems of premedical education.

When the American Medical Association and the Association of American Medical Colleges agreed on the 1951 Survey of Medical Education, it was decided to set up a Sub-committee to review pre-professional medical education. Dr. Aura Severinghaus was made the Chairman. This committee surveyed a representative sample of the colleges and universities of the United States and Canada, 115 in all, which offer acceptable and organized premedical training. The survey is now complete and last month a National Conference was held at Buck Hill Falls, Pennsylvania, to discuss the findings. During the coming months the Report will be published. It is more than an evaluation of the colleges offering premedical training by the Survey Committee; it is in a real sense a self-evaluation of the colleges, since the report will in large measure be an enlargement of the judgments made by the college representatives who attended the Conference.

Present plans call for a Second National Conference on Premedical Education to be held in the autumn of 1953, probably in Chicago. To that Conference will be invited representatives of all the colleges and universities which are approved for premedical education by the Council on Medical Education of the American Medical Association. Our purposes will not be to evaluate the Survey findings or to help construct the Report, as was the function of the Buck Hill Falls Conference. Our function will be to offer an opportunity for the discussion and study *of* the Report, and to disseminate the findings of the Survey as widely as possible.

We would like to provide an opportunity for at least one representative of every college and university in the land to become familiar with the Report, in order to discuss it with the faculties so that many of the weaknesses found in the colleges may be corrected and a better understanding be gained of the many problems of pre-professional medical education.

Numerous state-wide conferences on premedical education should be held in many states. Such conferences might result in study projects; experiments in premedical education suitable for individual schools; projects for intensive self-study by individual schools in the light of the Survey Report; studies of teacher effectiveness; of student evaluation; of grading standards for medical school admission, in which a premedical school would honestly compare itself with other schools; of advisory systems to discover what really *is* the best system of premedical training program for the individual school; of correct identification of courses in college bulletins so that a course in microtechnique is not labeled histology or a course in incubating eggs labeled embryology, and of general standardization of terminology and procedure in premedical training. These and many other problems need intense and objective study on individual campuses. Follow-up conferences should be held for evaluation of these studies and experiments. Let some plan be made now and some persons be authorized to arrange a follow-up conference to study the Survey Report in the premedical colleges of Texas and adjacent States to report on accomplishments in the light of the Survey's findings.

Encouragement for Service on the Health Team

That happy phrase, the Health Team, refers to the various ancillary medical services. It is pertinent here to examine two aspects of this important matter: one to emphasize the great need and opportunities for persons in the ancillary fields, and the other to make a plea for recognition of the people engaged in those fields.

The need for trained personnel in the various auxiliary medical fields is tremendous. Did you ever try to imagine a hospital in which there were no people except the patients and the

physicians? No businss personnel, no nurses, no orderlies, no pharmacists, no dietitians? Can you imagine medicine today without the drug manufacturers and salesmen; without the thousands of research workers in the employ of the drug manufacturers? Where would medicine be today without the thousands of teachers in the colleges, the medical schools, and the nursing schools? What advancements would not have occurred but for the work of the people in the basic sciences—anatomy, physiology, microbiology, biochemistry, and pharmacology, who are not medical doctors?

Medical practice today is *impossible* for just the physician and his patient. Besides that, there are the new fields of medicine itself where the most important people from the patient's point of view are not the physicians but the physical therapists, the occupational therapists, and trained nurses, people who give back usefulness and self-support and self-respect to the patient after he has survived his illness.

There is and probably will continue to be a great shortage of young women entering the study of nursing and willing to nurse sick people. There are at least 200 jobs—and good ones too—for every person now engaged in the study of physical therapy. About the same figures apply to those in occupational therapy. The research institutes and research departments of the pharmaceutical houses are seeking constantly for competent young people to enter the fields of medical research. Only a few hospitals have trained hospital managers because there are not nearly enough to fill all the jobs. Places for medical record librarians and medical librarians are unfilled in large numbers. These fields of medical service are crying for young people who wish to serve society, to contribute to the health of our people, and live satisfying lives.

Young people fail to appreciate these ancillary health fields for one or both of two reasons: either they do not know about these services; or they perhaps unconsciously resent the relatively low esteem in which people in these fields are held. No one wants to be thought of as a flunky; no one wants to be just the "hired help." Young people who have the high ideal of a career in medical service also wish the greatest and deserved reward of such service—the recognition of work well done and

pride of position. And it would cost so little! All it would cost is a small share of the prestige that accrues to the physician in the community. It certainly would be no great task to inform our students of the opportunities of service in these fields. But while it seems simpler, it is actually much more difficult to give them the recognition they wish and richly deserve. Medical doctors through the ages have stood with the priest in the estimation of the community, sometimes with less justification. There is a tremendous pride, and sometimes arrogance, among the members of the medical profession. For many of them it is impossible to share that pride of position with any other. Perhaps a little more humility on the part of the physician would be better fitting since he depends so much on nature for his cures, and would enable him to recognize his dependence on his many helpers in the health team.

This is a plea for encouragement of young people to consider a career in one of the services on the health team, not as substitutes for medical school but as a deliberate choice of a place to help people remake their lives after injury or illness. Even more let us seek for a generous recognition of the greatness of their services and a sharing of prestige with the physician, who so often must step aside for the therapist to take over and restore happiness and usefulness to the patient. Thus we would provide much needed members of the health team, improve the health of our people, and give these fine young people the recognition they so richly deserve. Let the medical doctors be reminded that true greatness is easily shared.

The Scientific and Humanistic Objectives of Premedical Education

The members of the medical profession are a highly individualistic group and the colleges of medicine are widely different and nearly as individualistic as the men who direct them. To satisfy them all in proposals for ideal premedical training is impossible. However, all seem to agree on the importance of maintaining both scientific and humanistic objectives. This is consistent with the concept of medicine as involving both scientific and artistic abilities.

What kind of a person is desired as a result of the premedical course of education? A composite reply to this question might be: A desirable candidate should have good physical health, reasonably pleasing appearance, ability to learn rapidly and retain well, ability to gain understanding as well as facts, sincerity of purpose, ambition, industry, dependability, unquestioned honesty both intellectual and social, a sense of humor, a high degree of adaptability, sound moral character, sound training in the scientific method, adequate information in the technical fields of chemistry, physics and biology, skill in language and use of his hands, a broad general education usually referred to as a liberal arts education, a sense of community responsibility and a knowledge of community problems in which he should sense his responsibility, a sound cultural and hereditary background, good judgment, and—mentioned by only a few—sound emotional development. A paragon indeed!

My remarks might end at this point. The qualities listed *are* the objectives of premedical education. But obviously such perfection exists only in the ideal. Certainly no twenty-year-old premedical student possesses all the characteristics mentioned. Yet we would all agree that those qualities are the desirable ones; the question then is, what is the minimum of these qualities the student *must* have to be considered qualified to study and practice medicine? Some of these qualifications may be considered further under three headings: the physical, the intellectual, and the emotional aspects of the candidate seeking admission to a college of medicine.

All agree that good health is essential for the physician both in training and in practice. But we should not confuse ill health with physical defects. Many medical students, for example, have completed the course of study in medical school while handicapped seriously. All of us know instances of successful medical students who had only one leg, who were color blind, who might have only one eye, or who had contracted tuberculosis and after being cured became active and successful physicians.

It is in the field of intellectual attainment in preparation for medical school that we place our greatest dependence. We look for high academic marks, strong recommendations on academic

ability, evidence of large knowledge in various fields,—unfortunately usually those closely related to medical subjects. In the field of the natural sciences and the methods of science, the results are usually satisfactory and the selection of students relatively easy. Among the different fields of scientific training the palm must go to the chemists. The chemists have succeeded better in training premedical students than teachers in any other field of science. Of course the chemists have more time allotted to them than is given to any other field. We agree with the allotment because the organic and physiological areas of chemistry have developed so much in recent years that it would be impossible to teach up-to-date biochemistry in medical school without the time spent in preliminary courses.

There are certain disadvantages that arise from the fact that chemists are such good teachers of chemistry. Their students are well grounded in chemistry and have the habit of exactness and the knowledge of specific qualities that identify specific substances. They have learned to describe by exact formulae what nature does when certain conditions exist in the chemical laboratory. Often such students suffer certain handicaps when they enter a laboratory in biology,—comparative anatomy for instance. There they discover that in animals nature seldom does the same thing twice; that each individual differs from all others, that no description of embryonic development or of adult structure will apply to all individuals of a species, in the manner that the formula for making sodium chloride from sodium hydroxide and hydrochloric acid *always* describes that process. The student must learn the rules of Science again—this time that in *living* things *variation* is the rule and that *his judgment* must be relied upon in interpreting his observations of nature. In my experience students doing "A" work in chemistry are often woefully weak in judgment though well informed in the facts of chemistry. Perhaps the chemists are missing an important point in education here—for of all things in medicine *judgment, based upon the facts of nature*, rather than the mere observation of the facts, is the most important. For in spite of all the statements to the contrary, the practice of medicine is not a science; it is an art—the art of applying the findings of science and *of all*

other experience to the health problems of that most variable of all creatures—man.

It is in the "experience other than science" that our premedical education is weakest. It is felt more and more in recent years that the physician of the future must be more broadly educated, that he must be able to take his place in his community as an intellectual and moral leader and know what he is talking about in more than just the field of medicine.

Underlying or resulting from this lack of broad education is the fact that so few premedical and medical students are really interested in *people* in the first place. They are interested in medicine; they avidly assimilate the facts of anatomy, pathology, physiology, diagnostics, and methods of examination and treatment: they learn to admire the "beautiful" case of brain tumor, of hypertension, of thyroid disease, but rarely do they learn or care to learn much about the person who is the "beautiful" case. Perhaps not being interested in the person who has the disease is a defense mechanism against damage to themselves psychologically. Perhaps it is the attitude that "if one looks only at the disease it will be just a disease; if one looks at the personality who has the disease one's own feelings will become involved." On the other hand it may be simply that they have been taught by men who themselves were interested in the disease but not the person. Students cannot be expected to reflect more desirable attitudes than their teachers.

In the psychological education or qualification for the study and practice of medicine, discrimination is much more difficult but also more important. It is in this area of the individuality that heredity and early family and other environmental influences have already left their mark. This is what the psychiatrists and psychologists call "personality." Personality means more than pleasing appearance and social behavior. It is the inner person, the unseen by others and often unseen by self. Just as man has carried with him many pre-human structural features that are now important in medicine, so he has carried with him a much larger number of cultural and psychological features that affect his behavior and ultimately his health. All of us carry them in some form or other and to greater or lesser

extent. These are the underlying motivations for much of our social and personal attitudes and patterns of behavior.

These primitive forces or drives are compelling, are often unrecognized, and are often unsocial or antisocial in nature. Psychiatrists are finding this a greater and greater source of the ailments of mankind. Psychiatry is one of the most rapidly growing fields of medicine, not because there are necessarily more people who develop mental disease but because we are just coming to realize the vast importance of mental and emotional factors in organic disease. To deal with these problems the student must have more understanding of the psychological causes of disease and a more sympathetic attitude towards those who are ill. To be able to do this he must have a wider and deeper understanding of human beings. This may to a large extent be gathered during his early years from his contacts with human life and emotions as seen in literature, art, history, philosophy, economics, and sociology, religion. He needs to observe humanity in its cultural settings. He needs to understand something of the vast interplay of forces in the social organism, to see individual and group motives,—desires, loves and hates, frustrations,—the constructive and destructive forces in action in society.

This is too large a task for a premedical student to gain firsthand. Therefore he must, and fortunately he can, gain much of this knowledge and understanding from the fields of study usually called liberal arts *providing* he has teachers who appreciate these things and who take the trouble to see, for instance, that the student reads Othello not only as great writing and great drama, but also as a penetrating study in human love and hate, the two strongest forces in life.

The exaggerated meaning of a word—a mere sound—may produce the most devastating states of mind and even mental disease. We are just beginning to realize the importance of language itself in individual and community health. For example, think how recently have the names of the common venereal diseases become socially acceptable either in polite conversation or the press. How recently also has the word "illegitimate" begun to cease wrecking lives. Great numbers of

neurotic and even psychotic persons have been made so by exaggerated brooding over something that has been call a "sin." Lack of emotional security, sense of inferiority, inability to accept failure all contribute to human disease and suffering. The attitudes relating to these problems are formed early in life and if better ones are to be formed we must begin early also.

Especially is philosophy to be urged in the premedical years. If philosophy is taught as a record of how men have tried to relate themselves to the rest of the world, as attempts to solve the eternal problem of psychological or spiritual security, as methods of acceptance and adjustment to the world, then philosophy must become the most compelling study in all one's educational experience. For that is just what philosophy is—the philosopher's answer to his *own* sense of insecurity and smallness in this great universe. Each of us must solve that problem in some way at some time. Without education we accept traditional beliefs. With mental growth these often become inadequate; new reasons for being and doing must be found. And they *must* satisfy the inner desire to find security and a feeling of value in the universe. In the child brought up in emotional security in the family the adjustment to adult situations is usually readily found. But in all too many cases this is not so, with the result that various forms of frustrations, neuroses, and even psychoses develop as attempts at solution of this fundamental need.

All this is to say that the physician must understand these things if he is to serve his people fully. The first requisite to that is to understand himself. He must himself be emotionally mature. Edward Strecker says:

"Maturity is a quality of personality that is made up of a number of elements. It is stick-to-itiveness, the ability to stick to a job, to work on it, and to struggle through until it is finished, or until one has given all one has in the endeavor. It is the quality or capacity of giving more than is asked or required in a given situation. It is this characteristic that enables others to count on one; thus it is reliability. Persistence is an aspect of maturity; persistence to carry out a goal in the face of difficulties, unpleasantness, discomfort, frustration, hardship. The ability to size things up, make one's own decisions, is a characteristic of maturity. This implies a considerable amount of independence. A mature person is not dependent unless ill. Maturity includes determination, a will to achieve and succeed,

a will to life. Of course, maturity represents the capacity to cooperate; to work with others, to work in an organization and under authority. The mature person is flexible, can defer to time, persons, circumstances. He can show tolerance, he can be patient, and above all he has the qualities of adaptability and compromise. Basically, maturity represents a wholesome amalgamation of two things: (1) dissatisfaction with the status quo, which calls forth aggressive, constructive effort, and (2) a social concern and devotion. It is morale in the individual."

Essentially maturity is the ability to accept the world and ones place in it; not with indifference but with realism. It is the ability to meet life's problems, face them, struggle with them, and either solve them or find a way around them. Not to be able to accept life as it is must eventually drive one to the self-created world of phantasy which is schizophrenia, or to suicide. Leading up to these final solutions are a multitude of neuroses with all the distressing physical and mental states related to them.

This kind of maturity is necessary in our future physicians. The complexities and uncertainties of our society are sending more and more people to their physicans with a large variety of symptoms which if properly interpreted are found to stem from the social structure and are reflected in the individual's health. The physician must be emotionally healthy himself; only then can he help others. Perhaps we cannot prevent some young people from remaining immature; it seems now unavoidable that many young people fail to achieve full emotional maturity. Certainly the emotionally immature are not satisfactory medical students or practitioners. Fortunately it is possible in most cases to detect the defective personality. Many intelligent laymen and any competent psychiatrist can detect the individual who is unlikely to reach full adjustment in his own life. The Rorschach and other tests, used by a competent person, give a most revealing picture of the personality. If these tests are not used routinely on all applicants, it should be used on all doubtful cases—that is, where there is any doubt as to the personality qualifications of the premedical student. They should be used only by a psychiatrist and the report should be simply favorable or unfavorable as regards admission. If a student is admitted against the advice of the psychiatrist, then that student should come under the observation and possibly treatment of a

staff psychiatrist as soon as he enters the medical school and his completion of the course should be partly dependent upon the studies and recommendations made by the psychiatrist during the medical course. This may seem unduly severe. But medical students have been damaged mentally and emotionally during and sometime *by* the study of medicine. Some deteriorate into alcoholics, some into drug addicts, and some even driven to suicide. Cases such as these justify heroic measures with all questionable individuals.

Thus it seems to me that our purposes, our objectives, in premedical education should include three aspects relating to the student:

(1) It should and must provide adequate training in the technical fields of the sciences, which is essential to enable the student to carry successfully the difficult program of the medical course.

2. Premedical education should also give an understanding and appreciation of the world, its history, and its peoples. Science should not be a tool only, however necessary that tool may be. It should also contribute to the student's attitudes and philosophy of life. From science may be gained not only skill but enjoyment, not only use but solace in the realization that in nature there are no accidents, no whims of fate, and the knowledge that what one finds in oneself or in one's patient today is the result of what happened yesterday and not the gift of chance.

3. The premedical course of study should also be a period of study *of* the student, his adaptibility, emotional stability, his "maturity" in another word, so that those developing satisfactorily and giving good promise of attaining maturity may be recognized. At the same time the frustrated, the emotionally maladjusted, the individuals with unsolved and perhaps insoluble problems of adjustment may be discovered and, if not provided with psychological treatment, at least be prevented from entering upon the study of medicine later to provide another example of the emotionally blind leading the blind.

Ideally this program should be tailored to fit each individual student. How long it takes to establish the technical fitness to enter medical school can be determined by his course work.

Whatever time is needed to prepare the student is worthwhile. It cannot take too long to prepare for the practice of medicine. It is a question of how much time we are willing to pay. In my view we should consider the *product*, not the *time*. If we develop the intellect but not the emotions, if we require information of our students but neglect wisdom, if we impart cold facts without the warmth of understanding, then we do not, and our physicians can not, serve our society fully.

ADVENTURES IN PROFESSIONAL HEALTH EDUCATION

H. G. Weiskotten

The early settlers in our country found themselves deprived of many of the comforts and opportunities enjoyed in the countries they had left. Not the least of these was facilities for education, especially at the professional level. Harvard University in 1636 and Yale in 1701, were founded primarily for the purpose of preparing young men for the Ministry. With the exception of a favored few who could travel abroad, an apprenticeship with a practicing physician was the only means whereby young men might prepare themselves for the practice of medicine.

In the latter half of the eighteenth century, the reputation of the European universities in medicine, especially the University of Edinburgh attracted many medical students from America. It was but natural that some of these men upon returning to this country should endeavor to develop institutions for the teaching of medicine. Thus in 1765, Doctors Morgan, Shippen and later Rush began in Philadelphia to teach Medicine, Surgery and Midwifery. This was the first medical college in the United States. Kings College in New York, organized its medical college in 1767.

The rapid growth of the population together with the westward migration created a demand for a relatively enormous number of physicians. Inasmuch as there were no legal restrictions on the establishment of medical schools, the number began to increase, although the medical apprenticeship did not entirely disappear, the lecture system became the generally accepted method of instruction. In many instances the faculty consisted almost entirely of itinerant teachers who travelled from school to school giving their courses of lectures. In most instances the entire medical course consisted of a series of lectures given over an eight to twelve week period in the Fall, usually with a repetition of the lectures in the Spring.

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Many of the medical schools were developed specifically for the financial gain of the promotors and the faculty. Some found them to be very profitable enterprises and with the increase in the number of schools, there developed a keen competition for students. In addition to these so-called medical schools, there developed what came to be called "diploma mills." These sold diplomas without attempting to provide any medical training whatever. No preliminary education, whatever, was required for admission of the medical schools of this period.

State control of medical practice during the early part of the nineteenth century came to be exercised through local medical societies and membership comprised sufficient qualification. Later a diploma from a medical school was accepted as an adequate credential.

Although a few of the schools were attempting to do a conscientious job, medical education was in a very chaotic state with little or no effort to maintain any standards whatever.

In 1847, the American Medical Association was organized with its main objective, the improvement of medical education and the medical care of the American public. One of its first activities was to appoint a committee on Medical Education. Although this committee was continued for more than half a century and each year endeavored to do something to improve medical education, it met with little success. Apparently one reason for this was the very great influence of the medical college professors in the affairs of the Association. Progress was so discouraging that at one of the meetings a resolution was presented to the effect that the attempt on the part of the Association to regulate medical education had signally failed in its objective, and had introduced elements of discord, and that any further interference would be useless and calculated to disturb the deliberations of the Associations. Fortunately, this resolution was not adopted.

Yet, this period was not devoid of progress. In 1871, Harvard Medical School adopted a three year graded curriculum and its example was soon followed by several of the better schools. An increasing number of schools began requiring a high school diploma for admission. The Association of American Medical Colleges, organized in 1890, recommended that all colleges adopt

this minimum standard for admission. Johns Hopkins in 1893 made a bachelor's degree a prerequisite for admission to its newly established medical school.

During the latter part of the nineteenth century great progress was being made in medicine and medical education in Europe, especially in the German universities. Much of this progress was in the newly established and rapidly growing sciences of physiology, pathology and bacteriology. American students returning from these foreign schools brought back a clear understanding of the importance of including in the medical curriculum these sciences which were basic to a sound medical education.

However, progress was so slow that in 1896 William Pepper, the elder of Philadelphia, in a review of medical education, stated that only five medical schools in the United States were offering satisfactory training for the practice of medicine.

In 1901, there was effected a reorganization of the American Medical Association, which brought all the local and state medical societies into an organic union as constituent parts of the Association. Thus, the Association was in a better position to make its influence felt throughout the country. There was immediately initiated an inquiry into the standards and policies of the medical schools. The need for a more comprehensive and continuing approach to the problem led to the establishment of the Council on Medical Education in 1904. Under the chairmanship of Doctor Arthur Dean Bevan the Council began developing an office and a staff which made possible personal visitation to the schools. These visitations called attention to the prevailing lack of standards, the inadequate physical plants, the meager resources and incompetent faculties in many of the then existing schools. Resentment of the Council's criticism on the part of substandard schools threatened to nullify the Council's efforts as it had the efforts of the Committee on Medical Education, for a period of a half a century. This led to the Council seeking the assistance of some outside agency with prestige in the field of education. Naturally and wisely the Council turned to the Carnegie Foundation for the advancement of teaching. At a conference in New York December 28, 1908, Doctor Henry Pritchett

of the Foundation agreed to undertake a survey of medical education throughout the country. It was agreed that in order to avoid any claims of partiality, no more mention should be made in the report of the Council on Medical Education than of any other source of information. It would then have the weight of an independent report by a disinterested body and would be published and distributed throughout the country. However, it was agreed that the Foundation would need to be guided largely by the Council's investigations. Thus it developed that Mr. Abraham Flexner of the Foundation, who was selected to make the survey, was accompanied in his visits to the schools by Doctor N. P. Colwell, Secretary of the Council on Medical Education.

The report of this survey published in 1910, and, written with the vitriolic pen of Mr. Flexner, aroused the American public to a recognition of the unsatisfactory state of medical education in this country. The report received wide publicity and could in no way be attributed to ulterior motives on the part of the Council on Medical Education. This report undoubtedly represents the most potent influence ever brought to bear upon medical education in the United States.

Strengthened by the Flexner Report, the Council, by repeated visitations to the schools and the annual publication of a classified list of the medical schools, was able to bring about a vast improvement in standards. At the same time, the Association of American Medical Colleges was gaining in strength and requiring satisfactory standards of accomplishment for membership.

Many of the weaker schools closed and others gained strength by university affiliation or consolidation with other schools. Very soon another potent influence was brought to bear on the efforts of the Council on Medical Education and the Association of American Medical Colleges. State boards of licensure were led to exclude from their examinations the graduates of the low grade medical schools which had continued to function. This resulted in the closing of additional unpromising schools.

Thus ended in large measure what have been referred to as the "policing" activities of the Council. From this point on the efforts of the Council were devoted largely to consultations, with

stimulation, advice and assistance in the continuing development of the schools.

True, it was deemed necessary to set up certain requirements as to subjects and hours to be included in the curriculum. However, as soon as they were generally adopted by the medical schools they were made less rigid and experimentation in the development of the curriculum and in teaching methods was encouraged. Each meeting of the Association of American Medical Colleges and of the Annual Congress on Medical Education sponsored by the Council on Medical Education has brought forth discussion of new experiments in curriculum content and methodology. With the elimination of the proprietary and substandard schools, the Council and the Association of American Medical Colleges as accrediting bodies, while always insisting on quality, have not desired or attempted to impose rigid quantitative standards. The great improvement in medical education in the past forty years has been largely due to the efforts of the administrations and the faculties of the medical schools themselves. Schools with unusually capable faculties and adequate resources assumed leadership in a variety of new developments which served as a stimulus to other schools. Career men in the various departments of the medical schools sought opportunities for the conduct of research in their special fields. Private contributors, public agencies and foundations were found to be willing to support such efforts and medical schools have become sources of many contributions to the advancement of medicine.

The great improvement in the teaching programs of the medical schools during this period has been largely due to the adoption of a simple but sound fundamental principle that is "Students learn by thinking and doing rather than by merely memorizing." It was this concept which resulted in experimentation in the development and continued expansion of the laboratory aspects of the basic medical sciences. It required a much more intimate association of student and teacher than had been customary. This same concept carried over to the third and fourth years of the medical course, led to an enormous decrease in the number of didactic and lecture courses and an ever increasing use of the hospital wards and the out-patient departments in the teaching of clinical medicine. This led to ever-in-

creasing responsibilities in the field of medical care being placed on the medical schools and the eventual development of Medical Centers in intimate association with them.

Medical educators have come to realize that all education is self-education and that all a medical school can hope to do is to provide the facilities, stimulus and guidance for students to educate themselves.

Fortunately, those responsible for the conduct of programs in the other colleges of the universities of the country are beginning to appreciate this concept in their educational efforts. If they are successful in applying it, future students entering medical school will have a much more satisfactory foundation than they have had in the past.

Although the importance of a period of training in a hospital between medical school graduation and practice had long been appreciated, previous to 1914, no medical school or state board of licensure required an internship. However, the value of the internship was recognized by the students themselves. Before any requirements whatever were initiated, 70% of the graduates voluntarily took internships before beginning independent practice.

The Council soon recognized the importance of satisfactory standards for this phase of medical education. It undertook a survey of hospitals engaged in the training of interns and in 1914, published its first list of hospitals approved for internship. Such approved lists have been continued and have served as a guide for graduates seeking internship training as well as a stimulus to hospitals desiring to maintain intern training programs.

All of these developments under the leadership of the Council occurred under the Chairmanship of Dr. Arthur Bevan with Doctor N. P. Colwell as full-time secretary. Upon the retirement of Doctor Bevan in 1928, Doctor Ray Lyman Wilbur, another great statesman of medical education, became chairman. Under his leadership the activities of the Council continued to expand in the fields of both undergraduate and graduate medical education. In the field of undergraduate education the most important development was a decision by the Council to undertake another comprehensive survey of all of the medical schools of the country. In the conduct of this survey the Council sought and

received the whole-hearted assistance of the Association of American Medical Colleges and the Federation of State Medical Boards of the United States. The objectives of this survey were entirely different from those of the survey conducted by Mr. Flexner and Doctor Colwell in 1909. With one or two exceptions the medical schools, often under serious handicaps, were making a sincere effort to maintain high standards. The handicaps under which the schools were operating differed in the different schools. In some instances they were merely financial. In others, they were in the administration of the university or of the medical school itself, or in the attitude of the faculty or of the local profession or local hospitals. The whole objective of this survey was to be of assistance to the medical school rather than to effect a discontinuance of any with the possible exception of two unapproved schools which seemed to offer no promise whatever. One of these closed immediately after the survey and the other a few years later. No publicity was given to the deficiencies found in the individual schools. The attention of all schools was called to advances in curriculum content and methodology which had been found advantageous. Many of the schools believed that this survey assisted them in solving many of their problems.

During this same period the Council was studying the trend toward specialization and the training of medical school graduates for the practice of the specialties. As early as 1913, it had surveyed the facilities for such graduate training beyond the internship. They had been found to be entirely inadequate. Proprietary graduate and postgraduate schools were offering entirely unsatisfactory brief courses for the preparation of medical school graduates for the practice of the specialties. There were no criteria for the recognition of satisfactory graduate training programs in the clinical specialties. Many graduates seeking to prepare themselves for the practice of the specialties were remaining on in the hospitals for advanced training following the completion of the internship. In 1923, there were adopted standards applicable to specialty training. However, it was not until 1927, that the Council undertook to publish a list of hospitals specifically approved for programs of training for preparation for the practice of the various specialities. These programs were designated as residencies.

Advances in the entire field of medicine have not alone resulted in a continuing increase in the number of specialized fields of medical practice but have resulted in enormous progress in the development of residency training programs.

Concurrently with these activities of the Council, the recognized leaders and national groups in the various specialties were considering the means whereby not alone the public but the medical profession itself could evaluate the competency of the practitioners in the various specialized fields. The Ophthalmologists of the country assumed leadership in the program and established the American Board of Ophthalmology. This board offered certification to those who had had adequate training and displayed competence in this field.

In 1933, the House of Delegates of the American Medical Association adopted a resolution authorizing the Council which had by then become the Council on Medical Education and Hospitals to express its approval of such specialty examining boards as conformed to the standards of administration formulated by the Council. To date there have been organized eighteen different American Boards in the constantly increasing specialized fields of practice.

Although this trend toward specialization in medicine and the development of the specialty boards has been frequently criticized, it represents one of the great advances in American Medicine. It has practically eliminated the inadequately trained and incompetent self-styled specialist. It has made available in an ever-increasing number of areas of the country unusually competent men in the specialized fields such as, for example, cardiac and brain surgery, previously only to be found in a few of the larger medical centers.

Thus, the Council on Medical Education and Hospitals has had a constantly enlarging problem in its efforts to assist in maintaining high standards in all phases of both undergraduate and graduate medical education. It has worked in close cooperation with the Association of American Medical Colleges and since 1942, there has functioned a Liaison Committee which meets several times each year for the purpose of co-ordinating the policies and activities of the two groups. Most actions relating to medical schools are taken concurrently by the two

groups after full discussion by the Liaison Committee. Representatives of both groups are represented in practically all visits to medical schools.

In their co-operative efforts, the Council and the Association of American Medical Colleges are now in the midst of another survey of medical education and have recently undertaken a new and major adventure in the field of medical education. The developments in all of the various phases of medical education have placed enormous responsibilities on the schools and have greatly increased its cost—so much so that it has recently appeared that the schools were facing a financial crisis. Our two groups with the assistance and support of the American Medical Association, a group of philanthropic foundations, a group of university presidents and a group of leaders in business and industry stimulated the organization of the National Fund for Medical Education. The Fund, under the Honorary Chairmanship of Mr. Herbert Hoover, is endeavoring to secure from private sources annual contributions for the continuing support of all the medical schools of the country. The American Medical Association has for the two years the fund has been in existence, contributed one-half million dollars per year to the American Medical Education Foundation which virtually serves as a subsidiary of the National Fund to secure contributions from the medical profession which are funneled through the National Fund to the individual schools. The program of solicitation of contributions from local medical societies and individual physicians is still in its infancy. The entire program offers promise and obviously, has enormous potentialities.

There are constantly other new and varied problems facing the medical schools of the country. However, given adequate financial support, under the leadership of the Association of American Medical Colleges and the Council on Medical Education and Hospitals of the American Medical Association, they will continue their contributions to the advancement of medicine and the improvement of the health and medical care of the American public.

A DEVICE FOR THE DECONTAMINATION OF BOTTLES WHICH HAVE HELD I^{131}-CONTAINING URINE

W. J. WINGO*

In connection with the determination of the state of thyroid function by the use of I^{131}, the collection and analysis for radioactivity of urine containing I^{131} is necessary. If the same urine-collection bottles are to be used repeatedly, it is essential for the accuracy of the radiochemical analysis that the bottles be thoroughly decontaminated after each use.

It has been found in this Hospital that the radioactivity of I^{131}-containing urine collected in soft glass bottles with the addition of carrier according to the method of Keating *et al.* (1) can be quantitatively removed (as determined by monitoring the open mouth of the bottle with an end-window β-counting tube) by repeatedly rinsing the bottles with tap water, filling them with tap water, and allowing them to stand for a week or longer before re-use. The repeated rinsing with tap water necessary to insure complete decontamination constituted a time-consuming and onerous chore, to avoid which the rinsing apparatus shown in Figure 1 was designed and constructed.

Tube *A* is a seamless steel tube, 1″ in outside diameter, bent to form a nearly complete circle as shown in the figure. Tubes *B* are ¼″ inside diameter iron pipes, welded into holes in Tube *A*. Other dimensions are as shown in the figure. All dimensions were chosen so that the bottles routinely used for urine collection (Abbott Laboratories liter infusion bottles and common gallon jugs) could be inverted over and be supported by Tubes *B* with the bottle necks clear of Tube *A*. The whole assembly is placed on an outdoor, direct connection to a sewer (C). Tube *A* is connected to the water supply by a valve and vacuum breaker.

In use, urine bottles are emptied into the drain in the center of *C*, and inverted over Tubes *B*. A moderate stream of water is turned on by means of the valve, and the bottles are left for

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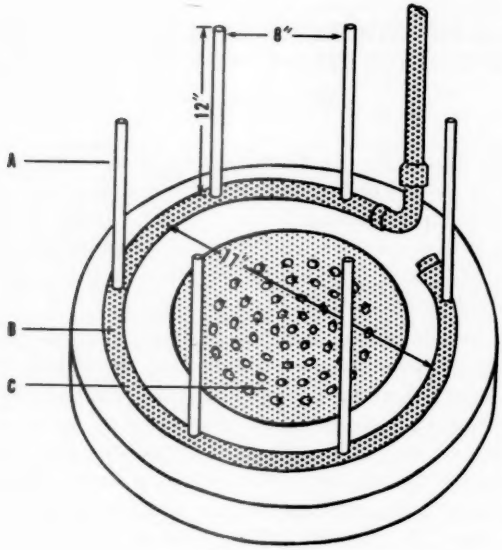

FIG. 1

5–10 minutes while being thoroughly and continuously rinsed by the water issuing from the open ends of Tubes *B*. This rinsing, followed by a soaking period of a week or more, is as efficient as the procedure formerly used in decontaminating the urine bottles and saves a considerable amount of time.

A smaller device operating on the same principle has proved useful in rinsing the sample bottles used in the determination of the I^{131} content of urine by a γ-counting procedure.

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AGE-WEIGHT-LENGTH RATIO OF AORTIC PARAGANGLIA IN THE ALBINO RAT

Ozro B. Wiswell*

While chromaffin tissue or scattered chromaffin cells occur throughout the body of almost all invertebrates and vertebrates, their anatomical characteristics and position have been but briefly discussed by various investigators. With regard to the accumulated chromaffin tissue at the bifurcation of the abdominal aorta, there is considerable disagreement among many investigators. Gerrish (1902), Piersol (1918) give the name "aortic paraganglionic bodies" (of Zuckerkandl) describing them as a group of chromaffin bodies which have developed from sympathetic system nodules. In human infants these bodies attain a large size, which may represent a fusion of several chromaffin bodies into an elongated cylindrical mass over one centimenter in length. They lie retroperitoneally, ventral to the abdominal aorta, at the level of the inferior mesenteric artery. Their size and extension are extremely variable and the "bodies" are said to undergo partial retrogression after puberty.

Many of the anatomically and physiologically significant features are not as yet well understood. Definite masses of chromaffin cells are usually closely associated with the sympathetic ganglia in the cervical, abdominal, and coccygeal regions of many vertebrates. Tissues where these cells are found in great numbers are particularly responsive histologically to stains containing chromic acid or its salts. When so treated according to Cowdry (1950) and Maximow and Bloom (1949), the small granules in these cells take on a yellowish, dark brown, or black coloration. In the opinion of many workers this coloration is brought about by the supposed presence of epinephrine in the cell. It is now recognized that an aggregation of these cells constitutes the adrenal medulla in mammals. Many animals have these cells grouped to form the aortic paraganglia (Zuckerkandl's bodies) which, in albino rats, are located on either side of the abdominal aorta at about the level of its bifurcation.

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While working on another problem involving necropsy of guinea pigs (Baldwin *et al.* 1943), it was observed that although these paraganglia were constant in region of occurrence, however, they varied in size, color, and topography. The presence of these variables stimulated a series of investigations to consider more fully their age-weight-length relation in the albino rat.

Materials and Methods

This report is based on observations of necropsis of 40 healthy albino rats, including both sexes, varying in ages from 5 days to 78 weeks. The animals in each age range were sacrificed at predetermined intervals and in all cases the chromaffin organs were found to be well vascularized, bilaterally placed and slightly dorsal and cephalad to the bifurcation of the abdominal aorta. They were also intimately associated with fibers of the sympathetic nervous system. The animals were sacrificed by ether anesthesia, weighed, and the aortic paraganglia measured in situ. The age-length data was compiled into eight separated group averages with five animals falling into each group. The age-weight data was compiled into seven group averages with six animals per group in five groups and five animals per group in two groups.

Observations and Discussion

In the young and middle-aged groups the aortic paraganglia were spindle-shaped, pearly white, glistening masses, in contrast to the adjacent irregular creamy colored masses of lymphatic tissue. Paraganglia in animals of the older age groups showed color changes to amber or brown, and the texture changes to a roughened, nodular character, with average adult organ dimensions of 7 mm. long and 1–2 mm. in diameter and in cross-section somewhat ovoid, thus indicating an approximate 5 to 1 ratio of length to diameter. A further point is that increase of length does not imply increase in diameter. Similarly, it should be noted that on histological survey[1] the chromaffin tissue of the normal adult organ increases as does the organ length and the proportion between the chromaffin tissue and organ stroma

[1]Further studies along this line are in progress.

remains the same. This survey, therefore, indicates that as the organ increases in length the amount of chromaffin tissue increases also.

With reference to the differences in the sexes, it was observed that the aortic paraganglia in females were always proportionally more elongated and apparently of a finer tetxure than in the males.

On the basis of aortic paraganglia length it seems probable that differential increments and decrements in growth are re-

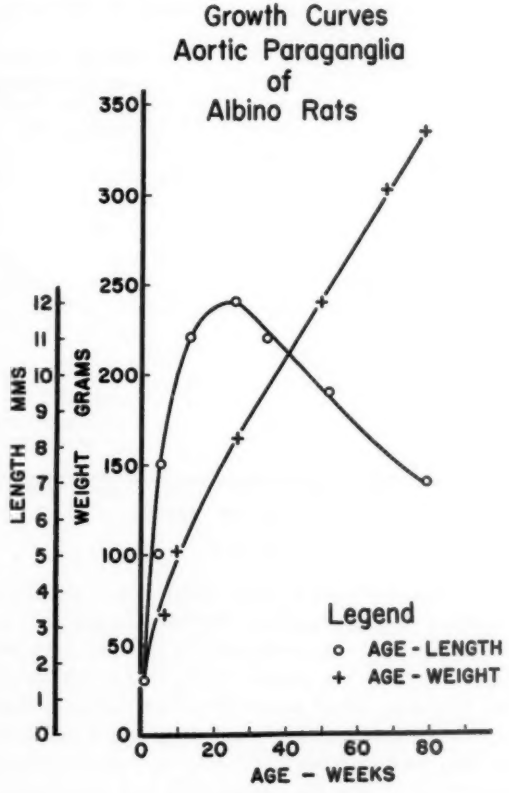

FIGURE 1

lated to chronological age and weight. Such growth differentials, length and weight to age shown in Figure I and then using the weight-age ratio as the movable baseline we compare the weight-length relationship to this in Figure II, thus indicate further significant age-weight-length changes. It will be noted that after albino rats reach the approximate age of 6 months, the length of the paraganglia diminish. Since rats of the colony from which these specimens were taken had a life expectancy of from 1½ to 2 years, it seems reasonable to assume that the apex of their physiologically active period is reached in about 8 months after which they can be considered as becoming old.

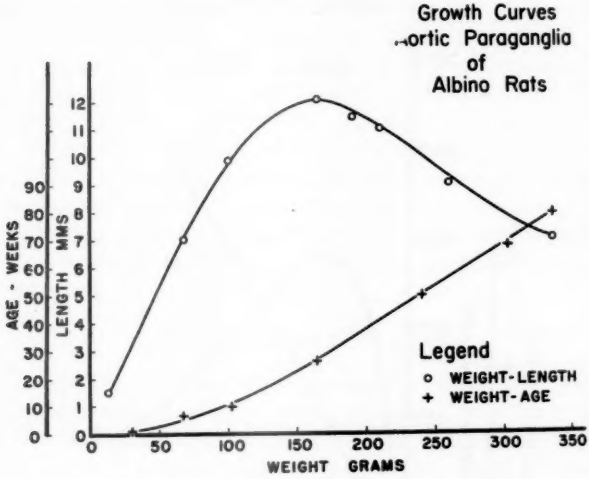

FIGURE 2

Young pregnant females upon autopsy present aortic paraganglia similar to paraganglia of the much older rats in length, color and topography. The constancy of this fact may well relate the paraganglia to a functional role in this condition. Five rats sacrificed five days after parturition had trilobate right paraganglia with an average of 15 mm. long and a single left paraganglia 8 mm. long. Autopsy of 3 rats, two months after parturition, gave evidence that the paraganglia had returned to normal size and color for their group.

Conclusion

1. The size, color, and topographical appearance of the aortic paraganglia change with age and physiological state of the albino rat.
2. Increase in size and change of color of the paraganglia accompanying pregnancy tends to indicate an endocrine relationship.

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